

PHOTOMICROGRAPHY

by

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The New York Microscopical Society*



SECOND EDITION

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Preface to the Second Edition

Advances in microscopy along many lines have been numerous since the publication of the first edition. New designs of microscopes, cameras, and accessories have made much earlier apparatus obsolete, even though basically the older equipment is in most cases just as efficient when properly used. The trend in photographic equipment has been largely toward universality of application, combined with automatic operation.

Among the advances in theoretical and applied microscopy can be mentioned phase and interference microscopy, electron microscopy, the widespread use of color photography, and the application of polarized light to many types of investigation not previously considered practical or useful. The introduction of Polaroid and its frequent substitution for the more expensive types of calcite prisms has helped to make the use of polarized light more general.

Much of the new apparatus has been so designed that a microscopist no longer need be trained in optical theory when it comes to the operation of his microscope or camera. Integral light sources, properly aligned for critical illumination, are now available in many forms for those who desire to use them. Flexibility in accessory apparatus, at least in the more elaborate equipments, enables one to do all types of microscopical work—light field, dark field, polarized-light, phase microscopy, and photography in color in all sizes of film from 16- and 35-mm. up to the capacity of the camera provided—all this with a complete range of magnifications—as well as copying, enlarging, reducing, and other ordinary types of photographic work occasionally required in photomicrographic work.

In the optical field there is now a definite trend toward the application of the method suggested by Sir Isaac Newton for eliminating chromatic and spherical aberrations through the use of spherical mirrors as objectives. It can be predicted that future developments along this line will be made and wider application of this type of objectives will result.

Another development in the sister art to photomicrography, microphotography, has come about as a result of the extensive employment of V mail during World War II. The advantage of reduced copies of publications, books, records, etc., to meet the evergrowing demands upon storage space has brought the art of microphotography into commercial prominence. This has resulted in the development of equipment for producing the reduced copies and means for facilitating the reading of them.

This new edition of Photomicrography has taken cognizance of these modern trends and illustrates some (it is not possible to include all) of the changes in design of microscopes and photomicrographic equipment. New chapters have been added to cover phase and interference microscopy as well as electron microscopy, which was in its infancy at the time of publication of the first edition. Other portions of the text have been added to and brought up to date when necessary. It has been the aim throughout to explain developments, where optical principles are involved, in terms comprehensible to individuals without technical training who may be called upon to use the latest equipment. Portions of the earlier edition which are still pertinent have been left unchanged. Illustrations of older equipment, now obsolete but still in general use, have also in several instances been allowed to remain.

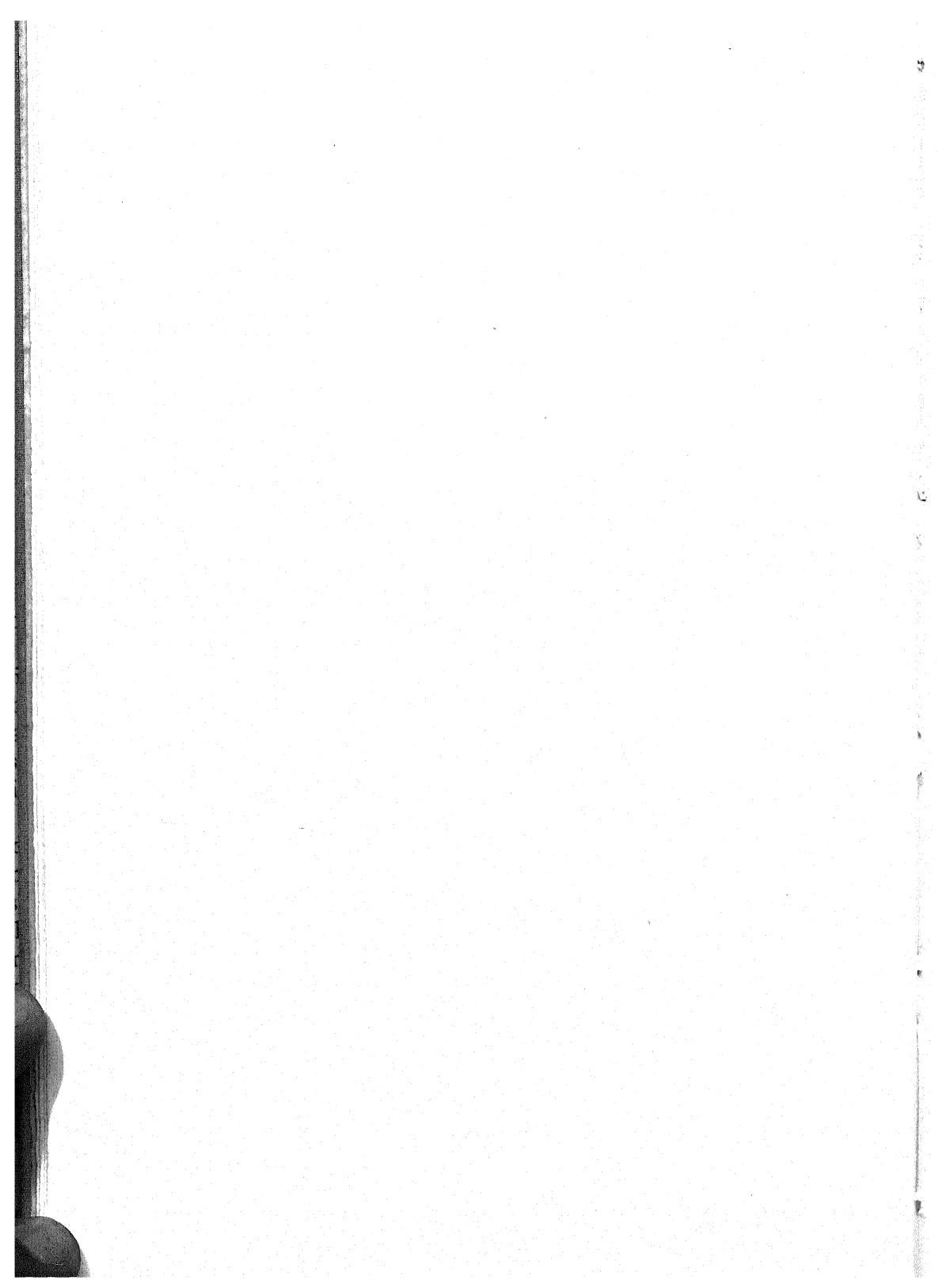
It should be pointed out that no attempt has been made to cover all the equipment manufactured or supplied by the many companies in this field. Much that has not been mentioned is of the highest quality. The deciding factor in determining what should be shown has been the publicity given in the past to manufacturers whose products have been in general use. Well-known British microscopes and equipment are unsurpassed in both mechanical and optical performances, and such names on a microscope as Watson, Baker, Beck (to mention a few) are guarantees of quality equal to the best; but these instruments are seldom to be found in use among microscopists in the United States. Those who do possess English instruments or optics (for the most part amateurs) are invariably enthusiastic in their praise of them. Yet for some reason the British manufacturers in general have made little or no attempt to invade the American market; hence these are given but brief mention.

Thanks are due to all the manufacturers and suppliers of microscopical and associated equipment (including the American agents for foreign companies) for their wholehearted cooperation with the

author in furnishing catalogues, information, suggestions, and photographs. These include Bausch & Lomb Optical Company, American Optical Company, Eastman Kodak Company, E. Leitz, Inc., Carl Zeiss (Zeiss-Winkel), Ercona Corporation (East German Zeiss), Wm. J. Hacker & Company (agents for Reichert), the R. Y. Ferner Company (U.S. representative for Cooke, Troughton & Simms), Silge & Kuhne, Photovolt Corporation, Fish-Schurman Corporation, Fisher Scientific Company, Radio Corporation of America, Philips Electronics, Inc., Farrand Optical Company, and many individuals in the various companies who have given personal help.

September 1958

ROY M. ALLEN



Preface to the First Edition

Rapid modern advances in the application of the microscope to problems of all sorts, both research and commercial, have naturally resulted in a simultaneous development of the art of photomicrography. The latter can no longer be classed as a hobby, indulged in only by a small group of amateur microscopists; it has become an essential adjunct to the use of the instrument itself in practically every scientific and commercial field.

In spite of this, authoritative reference books on the subject of photomicrography are lacking. Whether this is the cause or the effect of a common idea that expert knowledge is unnecessary for taking pictures through the microscope is immaterial. The fact remains that photomicrographs (of a sort) are being taken daily by hundreds, if not thousands, of microscopists, in every line of microscopic endeavor, whose sole qualification for the task is a fundamental knowledge that the image formed by a microscope can be projected onto a sensitive photographic film and be reproduced thereby.

That better pictures can be taken as one's experience and knowledge of the technique increase is to be assumed, as is also the fact that the majority of individuals can learn more rapidly and easily by assimilation of the published experiences of others than by the slower process of personal plodding. This is the reason for the existence of this book.

Although the close relationship between visual work with the microscope and photomicrography would seem to have warranted inclusion of the latter in the author's book, *The Microscope*, this was not feasible because of the large amount of material which would have had to be included to cover the subject in a thorough manner. The alternative was the complete segregation of the two subjects and their publication separately.

A work dealing primarily with the photographic aspects of microscopy cannot, of course, undertake an exhaustive discussion of the elementary optics of the microscope to the same degree as a book primarily on the instrument itself. Some knowledge on the part of the microscopist must be assumed. On the other hand, it is desirable that a work on photomicrography be sufficiently self-contained to be understood without constant reference to outside matter. This implies

that some duplication of subject matter common to both visual and photographic work must occur — so much, at least, as may have a bearing on the production of ideal photomicrographs.

The material contained in the present volume is derived almost exclusively from the author's personal experience, which has extended into practically every known application of the microscope, and is passed on in the hope that it will prove for many a substantial short cut to a working knowledge of photomicrography.

For the benefit of those already familiar with the use of the microscope, the text has been so arranged that only matters of direct interest need be considered, without wading through irrelevant matter. On the other hand, beginners can follow through each chapter in the sequence presented, and after becoming acquainted with the subject from the standpoint of basic principles and the various ways these have been worked out in mechanical design, can take up the practical technique of photomicrography. The chapter on photographic processes has been added to provide information on this phase of the work for those who may not have had previous experience with them.

I wish to express my appreciation of the wholehearted cooperation extended to me by various companies in providing cuts of the various apparatus and equipment shown. These include, The Bausch & Lomb Optical Co., Spencer Lens Co., E. Leitz, Inc., Carl Zeiss, Inc., Eastman Kodak Co., Radio Corporation of America, Cooke, Troughton & Simms of York, England, and others.

R. M. A.

June 1941

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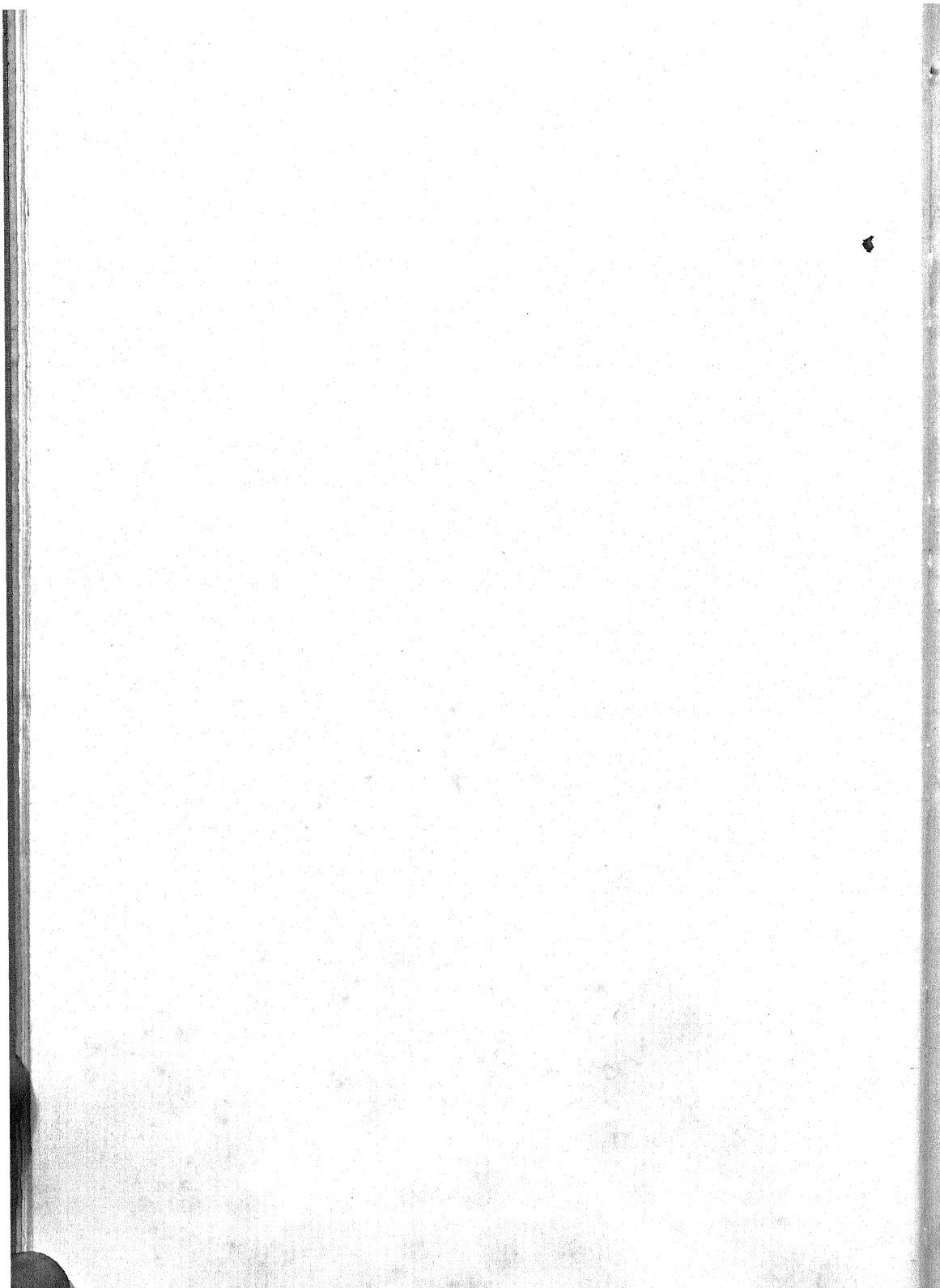
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Fundamental Principles of Photomicrography

Definitions

The word *photomicrograph* is a compound of *photograph*, a picture produced through the instrumentality of light, and *micrograph*, an enlarged reproduction of a minute object. Originally "micrograph" meant only a drawing, made by free hand, or by means of a pantograph, as these were the only methods known for producing the enlarged image. The application of photographic processes of reproduction to the microscope provided a new method of securing enlarged pictures of minute objects, hence the combined word *photomicrograph*. This compound word necessarily is limited to mean a picture taken through a microscope, by means of light acting on a sensitive emulsion, but the word *micrograph* used alone has had to be broadened in meaning to include pictures of minute objects either drawn by hand or produced through photographic processes.

As a matter of fact, photomicrography is so rapidly replacing hand drawings (made by means of a camera lucida attached to a microscope) that it is probably only a question of time until the term *micrograph* will come to mean *only* a photomicrograph.

Another term introduced to cover a particular class of photomicrographs is *photomacrograph* (incorporating the Greek word for "long," *makros*), meaning a photographic image of a relatively large object magnified only a few times, i.e., not over ten diameters. A "macrograph" is, then, a *slightly* enlarged picture of an object.

Sometimes the word *microphotograph* is used for photographs taken with the microscope, but this word is used incorrectly, for it means "a minute photograph," which must be examined as a microscopic object, under the microscope, in order to observe the details of the picture. The relation of microphotographs to the microscope, and the method of producing them, will be considered in Chapter 8.

Relation of Photomicrography to Ordinary Photography

The possibility of taking pictures by means of the microscope is dependent upon the fact that basically there is no difference between a camera lens and a microscope. They both obey the same laws of optics in producing an image of a properly illuminated object. As a matter of fact, photomicrography and ordinary photography merge so perfectly into each other that it is difficult to say just where one leaves off and the other begins. The best place, therefore, to commence a discussion of the optics of photomicrography is with the camera lens.

In every case, a camera lens must be of what is known as the "positive" lens type, i.e., one that is thicker in the center than at the periphery. Only a positive lens (the term of course includes compound lenses functioning together as a single positive lens) can produce a *real* image such as is required for photographic purposes. Negative lenses are those which are thinner through the center than at the periphery. These, or combinations of lenses which have a resultant effect similar to a negative lens, cannot produce a photographic image. The image in this case is known as a *virtual* image.

When an object is located a great distance from a camera lens, ordinarily known as "infinity," the rays from the object are brought together by the lens to form an image of the object in what is called the focal plane of the lens; the distance of this plane from the optical center of the lens is designated the *focus** of the lens. For all practical purposes, the distance "infinity" can be considered as anything over 200 times the focal length.

The size of the image bears a definite relation to that of the object, which is the ratio of the distances between the lens and the object, and the lens and the image. Thus the image of an object located 200 times the lens focus from a camera will be $1/200$ actual size. Only when the object is located at infinity will the image plane be distant from the lens the exact focal length of the latter. As the object comes nearer, the image plane moves farther away from the lens, and the ratio between the object size and image size changes accordingly. This, of course, is why cameras must be focussed for the proper object distance, if one is to obtain sharp pictures.

* Technically, this is known as the *principal focus*. Actually, every lens has two foci, one on each side of the lens. These are called *conjugate foci*. The conjugate focal point corresponding to the principal focus is located at infinity. As it comes nearer the lens, the corresponding conjugate focus recedes.

When the object is brought to a certain distance from the lens it is found that the image plane has receded to a point where it is the same distance from the lens as the object distance. This distance is found to be exactly twice the focal length. Under this condition, the image size is identical with the object size. With ordinary hand cameras this cannot be accomplished, because not enough adjustment or bellows length is provided; therefore, if photographing an object full size is desired, a special long-bellows camera is necessary.

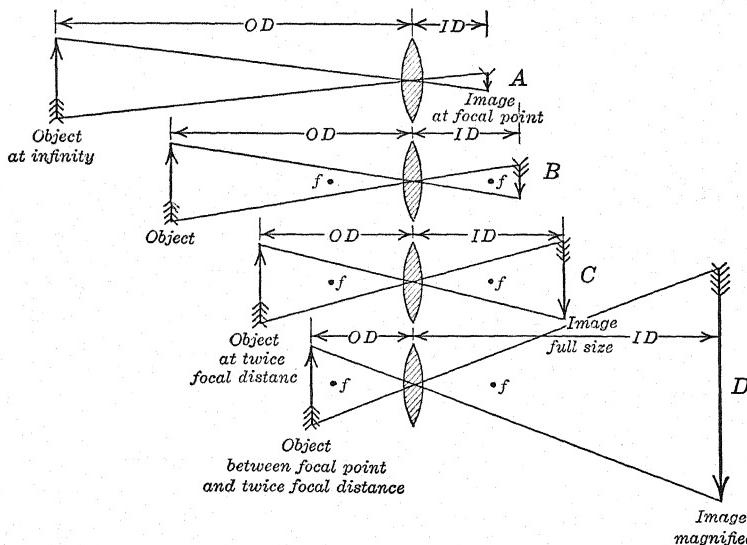


FIG. 1. Relationship between Image size and Object size. ($I : O :: ID : OD$)

If the bellows is sufficiently long, it is not obligatory to stop when full size has been reached; bringing the object still nearer results in moving the image plane farther away and the image then becomes *greater* than the object, or *magnified*. Thus the same camera lens becomes in effect a photomicrographic lens producing possibly a magnification of several diameters. These various conditions are shown diagrammatically in Figure 1.

Thus far we have not taken into account the effect of a change in the focal length of the lens on image formation. Here the parallel between a camera lens and a microscope becomes still closer. Let us study a few examples and apply the simple mathematical ratio to the object and image size.

Assume an object 10 inches high located 100 feet distant to be

photographed by a camera equipped with a 6-inch ($\frac{1}{2}$ ft.) lens. Its photographic image is $10'' \times \frac{1}{200} = \frac{1}{20}$ of an inch high. Using a 12-inch (1 ft.) lens under the same conditions, the image size would be $10'' \times \frac{1}{100} = \frac{1}{10}$ inch. In other words, when the distance is fixed, the size of the image can be increased substantially in proportion to the increase in the focal length of the lens employed.

This will hold more or less true as the distance of the object from

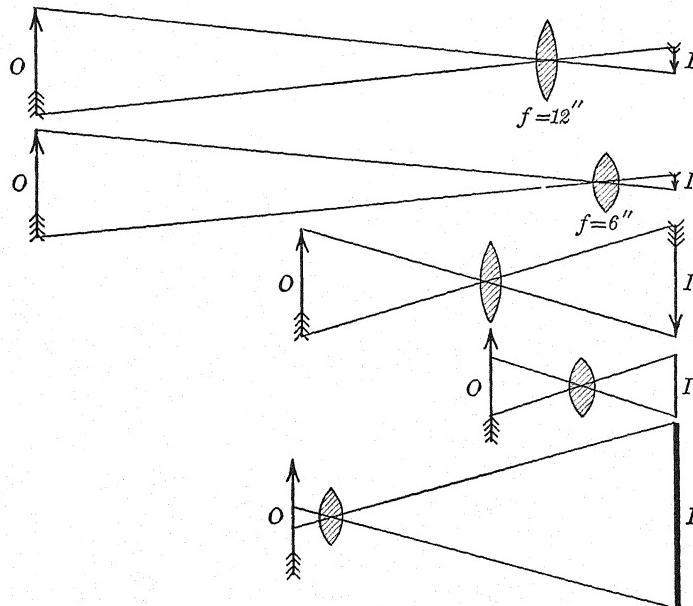


FIG. 2. Effect of the focal length of the lens on image size

the camera is decreased. But with extreme nearness of the object other factors enter the problem. We have already seen that any lens is capable of producing a full-sized image when the object is distant twice the focal length of the lens from the latter. With a 12-inch lens this occurs when the object is 24 inches away. It also requires a camera bellows length of 24 inches, while with a 6-inch lens the object yields a full-sized image with an object distance of 1 foot and only a 12-inch bellows length is required. Should we wish to make a several times enlargement of the object we would discover that a 12-inch lens is almost prohibitive because of the excessive bellows length required for the camera; the 6-inch lens is far superior in this respect. Assum-

ing that our maximum bellows length is limited to 36 inches, it is evident that employing a still shorter-focus lens, say a 3-inch, would enable a much higher magnification to be obtained. Going to a $\frac{1}{2}$ -inch lens would provide a possible magnification around 70 diameters. This has no bearing at all on the fundamental law; it only means that should we wish to secure a similar magnification with a 12-inch lens, we would require a bellows in the form of a tunnel about 75 feet long.

Conditions arise both in ordinary photography and photomicrography where lenses of longer focus are required to meet certain conditions. These conditions, as will be pointed out later, have to do with the relative area of the object which must be included in the photograph; the longer-focus lens accommodates a correspondingly greater area. Figure 2 illustrates graphically the effects of variation in the focal length of the lens on the image size and the way in which the area of the object included in the field of view is restricted by the use of shorter focus lenses.

A question might naturally arise in connection with the statement already made that a $\frac{1}{2}$ -inch lens used with a bellows extension of 3 feet will yield a magnification of some 70 diameters: "Why not use a still longer bellows and obtain magnifications of 100 or even several hundred diameters, with the same lens?" The answer to this question provides the justification for introducing the microscope and microscope lenses into the picture. It might appear sufficient to dismiss the suggestion of magnification by means of a long bellows wholly on the basis of its mechanical impracticability, but this is not the primary objection to it.

In the first place, the $\frac{1}{2}$ -inch lens proposed is already out of the class of ordinary camera lenses and can properly be considered a microscope lens. There is, in this respect, a considerable overlapping in the region of short-focus camera lenses (even excluding motion-picture cameras) and long-focus photomicrographic lenses. The former extend down to less than 2 inches and the latter up to at least 4 inches (100 mm.). There is, however, as will be demonstrated later, usually a radical difference in the design of the lenses for the two purposes.

In the second place, a more obvious solution would be to use the same bellows and reduce the focal length of the lens. A $\frac{1}{4}$ -inch lens used on a 36-inch bellows would double the magnification obtainable with a $\frac{1}{2}$ -inch lens. This is the basis of the actual procedure employed in the production of highly magnified photomicrographs. Merely shortening the focal length of the lens, however, would not be satis-

factory; there are certain optical laws inherent in the problem of producing magnified images of an object in which structural details are to be revealed in direct proportion to the amount of enlargement secured. An understanding of these laws and the manner in which they are met in the design of microscope lenses is essential to a thorough knowledge of photomicrographic technique. But before they can be taken up a discussion of some fundamental optics is in order.

Spherical and Chromatic Aberrations in Lenses

Unfortunately a positive lens constructed from a single piece of glass is not satisfactory for either high-quality photographic or photo-

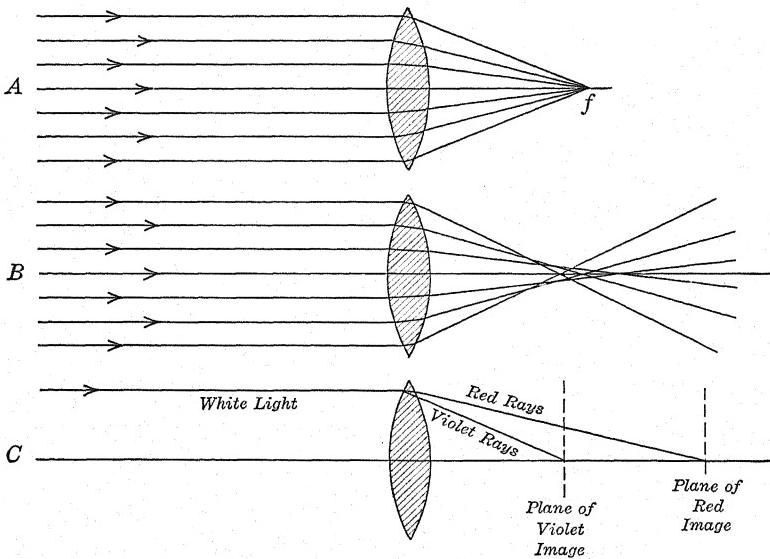


FIG. 3. Spherical and chromatic aberrations

micrographic work. This is because certain aberrations are present which interfere with the production of a perfect image.

The ideal condition is represented in Figure 3A, where all the rays proceeding from a point source of light located at infinity are brought together to form a point image. But what actually happens is that the rays passing through the outer zones of the lens are refracted excessively so that they come to a focus nearer the lens than those through the central zone. This effect, shown in Figure 3B, results

from the fact that lens surfaces are ground as segments of spheres. It is known as *spherical aberration*. When the image of a point is no longer a point but a fairly large circular area, the image of a concrete object, which should be conceived of as composed of an infinite number of points, cannot possibly be sharp but will be fuzzy in proportion to the amount of spherical aberration present.

Then again, white light is not a single entity but is composed of rays vibrating at different frequencies, or, in other words, possessing different wave lengths. These different wave lengths affect the eye differently; the sensation experienced is what we know as color. The entire series of wave lengths affecting the eye, and which are largely responsible for the production of a photograph, is known as the spectrum. It extends from the shortest wave, the violet (about .4 micron in length) * through indigo, blue, green, yellow, orange, and red (about .7 micron wave length). The spectrum is familiar to all from its common occurrence in nature as the rainbow.

When white light, containing these various wave lengths, is passed through a lens and refracted,† it is found that each different color is refracted differently; those at the violet end are bent considerably more than those at the red end. This fact explains the rainbow, which is caused by sunlight passing through raindrops, each of which functions as a minute spherical lens, thus refracting the color differently. The separation of the various wave lengths, due to the difference in the amount they are refracted, is known as *chromatic aberration*. It is illustrated in Figure 3C.

If monochromatic light (i.e., one particular wave length only) were employed for photographing, chromatic aberration would not interfere with securing a sharp image, but as the focal length of the lens is different for each wave length, it must be focussed for the particular color used. Because the focal length of the lens varies with the wave length, the magnitude of each color image is, of course, different, so that when they are all superimposed in one exposure, the resultant image is poor.

Fortunately, optical glass of great diversity in both refractive index

* The micron is $1/1000$ of a millimeter, roughly about $1/25000$ ".

† The deflection or bending of light rays occurring when they pass from one medium to another, such as from air into glass (or the reverse), is called *refraction*. The unit of measurement of refraction is known as the *index of refraction*, air being considered unity (1.00); the refractive index of ordinary glass runs around 1.5. The difference in the refractive index of glass for different wave lengths of light is its dispersion.

and dispersion is available and through combinations of positive and negative lenses made from these glasses, it is possible to produce a compound photographic lens, whether of camera or microscope type, in which both spherical and chromatic aberrations are practically eliminated. In such lenses, rays of light of all colors pass through all zones from center to periphery to meet at substantially a point, as in Figure 3A.

Even assuming perfect compensation for both spherical and chromatic aberrations, the vibratory nature of light itself prevents the formation of an absolute point image of a theoretical point source of light. The image inherently possesses a positive circular area. The condition is illustrated in an exaggerated manner in Figure 4. The circular disc image of the theoretical point is called the *circle of confusion*.* It is aggravated in size when spherical or chromatic aberrations are less perfectly corrected, but the determining factor in establishing the theoretical minimum diameter for any given lens sys-

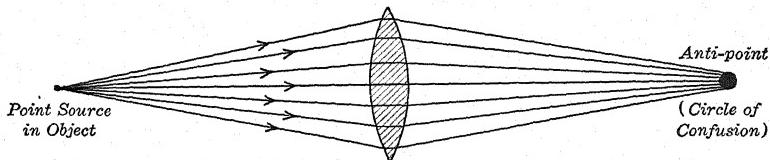


FIG. 4. The image of a point, formed by a lens, is never a point, but a circular disc of definite diameter, known as the anti-point, or circle of confusion

tem is the wave length of the light employed. The diameter of the disc is reduced as the wave length decreases. Or, stated in another way, sharper pictures can be secured with the same lens by using blue light than are obtainable by using red.

From a practical standpoint, for any given wave length, when enlarged images are involved, the greatest single factor in reducing the size of the circle of confusion is the angular aperture of the lens. In Figure 5, two lenses are shown diagrammatically which are assumed to be alike in every respect except that the angular aperture of *B* is twice that of *A*. Or, to state it more accurately from a technical standpoint, the trigonometric sine of one-half the total angle of the limiting rays entering *B* is twice that of *A*. Then the antipoint, or circle of confusion, of the former is one-half as large as that of *A* and the ability to resolve detail is accordingly twice as great.

* From the microscopist's standpoint the "circle of confusion" is often called the "Airy disc" or *antipoint*.

In respect to the relative value of large-apertured lenses to camera work and photomicrography there is this fundamental difference. In the case of the former it is primarily of advantage to secure greater light-gathering power, usually referred to as the "speed of the lens."

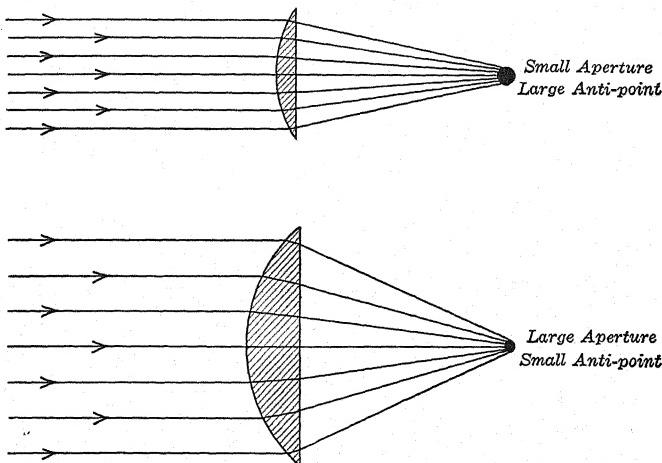


FIG. 5. Decrease in size of the anti-point with increase in the aperture of the lens

This is expressed in terms of the ratio of the effective diameter of the lens to its focal length, the term $f:4.5$ implying a focus for infinity equal to 4.5 times the largest diaphragm opening. With the diaphragm on the same lens closed to one-half the diameter (i.e., set at $f:9$) the light would be diminished to one-fourth the intensity, and consequently an exposure four times as long would be required.

In photomicrography, the object is usually stationary and the length of the exposure is therefore immaterial. What we are interested in is seeing more structural details. Indeed, in proportion to the extent of the enlargement secured, this is the only reason for wanting a magnified image in the first place.

Thus the use of a greater aperture in microscope lenses is only incidental so far as the light-gathering power is concerned (although of value in high magnifications, as otherwise unduly long exposures might be necessary); the prime importance lies in the increased ability to see greater detail. The most convenient way of expressing this characteristic of a microscopic lens is therefore not in terms of f ratio, but as *Numerical Aperture*. This term was originated by Ernest Abbe to express the sine of one-half the angular aperture, multiplied by the

refractive index of the medium with which the lens is designed to operate. It is written

$$\text{N. A.} = n \sin U,$$

in which N.A. is numerical aperture, n is the refractive index of the medium, and U is one-half the angular aperture. With all lenses designed to work in air, n is equal to unity and hence actually plays no part in the equation.

The limit of enlargement of which any lens is capable can be demonstrated mathematically, basing the figures upon the size of the circle of confusion. Beyond this limit further increase in image size results only in *empty magnification*, i.e., magnification without corresponding increase in resolution of structural details.

This limit is usually expressed as roughly 1000 times the N.A., for the optical center of white light (around .55 micron). Thus a camera lens* which has an N.A. (equivalent of f:4.5) of .11 would have a theoretical limit of magnification of 110x irrespective of its focal length. As such magnification is a low power from a microscopical standpoint, one limitation upon obtaining highly magnified images by the expedient of indefinite increase in bellows length is evident.

But there is still another very important factor to be taken into account, which affects the application of ordinary camera lenses, no matter how highly corrected they may be, to photomicrographic purposes.

As already pointed out, corrections for spherical and chromatic aberration involve the use of compound lenses, often made up of several components. Figures 6, 7, and 8 illustrate some of the well-known camera lenses on the market. One characteristic common to two of these lenses (the Goertz Dagor excepted) is that they are not symmetrical when viewed from the front and back. In ordinary photography there is an additional condition to be satisfied, known as astigmatism. It has been found that to overcome this, as well as spherical and chromatic aberrations, and at the same time provide fast lenses at a reasonable cost, unsymmetrical combinations offer one of the best solutions. Hence many of the well-known camera lenses are of the nonsymmetrical type. But to obtain the best results an unsymmetri-

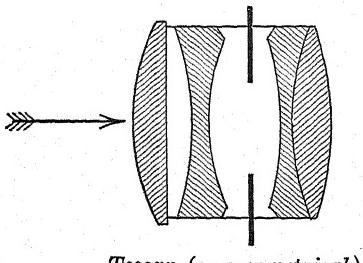


FIG. 6

symmetrical when viewed from the front and back. In ordinary photography there is an additional condition to be satisfied, known as astigmatism. It has been found that to overcome this, as well as spherical and chromatic aberrations, and at the same time provide fast lenses at a reasonable cost, unsymmetrical combinations offer one of the best solutions. Hence many of the well-known camera lenses are of the nonsymmetrical type. But to obtain the best results an unsymmetri-

* But corrected for the object to lie in the plane of the shorter conjugate focus.

cal camera lens must be used in the way it was designed to be used — that is, the front of the lens must always face toward the *longer* of the conjugate foci. The shorter conjugate focus is always that next to the film. As a result of this, unsymmetrical lenses designed for distant

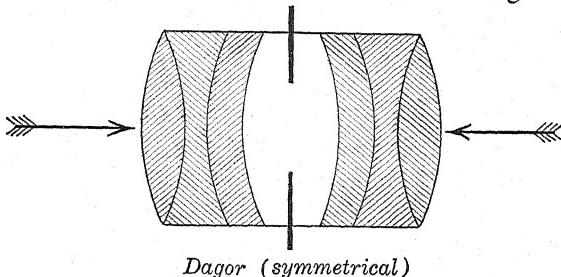


FIG. 7

photography are not even ideal for copying at full size and produce an inferior image when the longer conjugate focus is at the back of the lens. In a symmetrical lens, such as the Dagor, Figure 7, it is obvious that either the front or back could be toward the longer or shorter conjugate focus without affecting the operation. Hence, if camera lenses are to be employed for taking low-power photomicrographs, one should make sure they are of the symmetrical type, of which there are several on the market. As an alternative, it often suffices to reverse an unsymmetrical lens for enlarging purposes, so that the light from the object enters the back — i.e., the front of the lens is turned toward the film.

Manufacturers of long-focus lenses intended especially for photomicrography compute them for this purpose, so that naturally they are usually superior to camera lenses of equivalent focus and aperture.

It is a fundamental principle, well known to all camera enthusiasts, that lenses with large apertures possess correspondingly less depth of focus, especially with nearby objects. If one focusses on a person only a few feet from the camera, everything in front and back is out of focus. When greater depth of focus is desired, we resort to "stopping down" — that is, closing the diaphragm until the desired effect is obtained. In other words, we work with a lower aperture. This principle

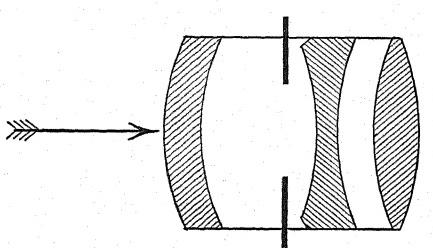
*Sigmar (unsymmetrical)*

FIG. 8

applies to photomicrography as well: an increase in the N.A. reduces the depth of focus, although to the benefit of greater resolution. In low-power photomicrographic lenses use is made of an iris diaphragm for this purpose, just as with camera lenses, but with objectives of higher power the same end must be reached by the substitution of another lens of lower N.A.

As a result of this possibility of securing either an approximation of an optical plane or an appreciable depth of focus, at will, the trained photomicrographer possesses a means of meeting a wide range of conditions often present in microscopical problems.

The Compound Microscope

Thus far, in establishing a relationship between photomicrography and ordinary photography we have assumed merely the employment of suitably corrected lenses of diminished focal length and increased aperture, for securing high-power photomicrographs.

In theory this is possible, even to the highest power, but in practice it becomes necessary to obtain extremely short-focus lens equivalents by compound magnification through a microscope.

Low powers require single-lens magnification only, and for this range it is not even necessary to employ a microscope. Lenses for this purpose generally start at about 100 mm. (4''); then a complete series is available, such as 75 mm. (3''), 50 mm. (2''), 35 mm. (1½''), 20 mm. (4/3''), and 16 mm. (2/3''). At this point we reach the realm of the compound microscope. As the name implies, the compound microscope effects its ultimate magnification by remagnifying an already magnified image. The lens nearest the object, designated the objective, functions just as would a camera lens arranged in the manner described for producing an enlarged image of an object. This enlarged image in turn becomes the object on which the second lens, called the eyepiece, or ocular, is focussed. The image formed by the second lens is the one projected onto the sensitive film to produce the photomicrograph. This is shown diagrammatically in Figure 9.

As the compound microscope is an essential component of all high-power photomicrographic equipments, we will pause for a brief study of its mechanical design, a simple model of which is shown in Figure 10 with the various parts designated.

The objective and eyepiece are mounted on the ends of the body tube, the former by screwing in place, the latter merely by a sliding fit.

Thus either can be removed at will and one of different magnifying power substituted. Practically all manufacturers now make their regular series of eyepieces to the same standard diameter (23 mm.). Also, the screw thread on the bottom of the tube, known as the Royal So-

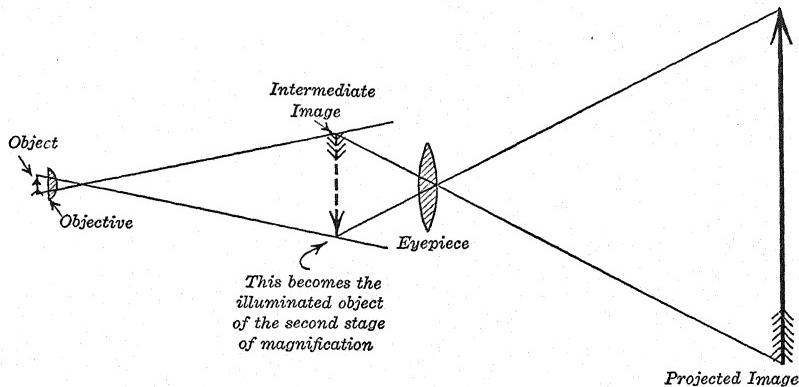


FIG. 9. Schematic diagram of the compound microscope

cietty thread, has been adopted by all manufacturers for both the microscope tube and objectives. Hence not only all objectives and eyepieces made by any one company are interchangeable, but they can be interchanged with those of other manufacturers as well.*

In one respect, however, complete interchangeability is limited by standardization of different tube lengths for the microscope. Some makers have adopted 160 mm. as the distance between the shoulders against which the objective and eyepiece rest, known as the mechanical tube length. In other makes 170 mm. is standard, while microscopes intended for metallurgical purposes have tube lengths ranging from 190 to 215 mm. This variation in tube length limits interchangeability of the optical parts due to the fact that objectives are computed to give perfect results only when used with a definite tube length. For this reason it is always necessary to make sure that the tube length of the microscope is correct for the particular lens employed. This can usually be done by an adjustment of the draw tube into which the eyepiece fits, and which in turn slides into the main part of the body tube.

The objective and eyepiece, together with the body tube, constitute

* There are a few exceptions to this general statement, as in the case of special microscopes where a larger-diameter eyepiece is employed, and low-power photomicrographic lenses of large aperture which of necessity are larger in diameter than can be accommodated by the Royal Society thread. Also, many microscopes of older vintage will be found that employ larger-diameter eyepieces.

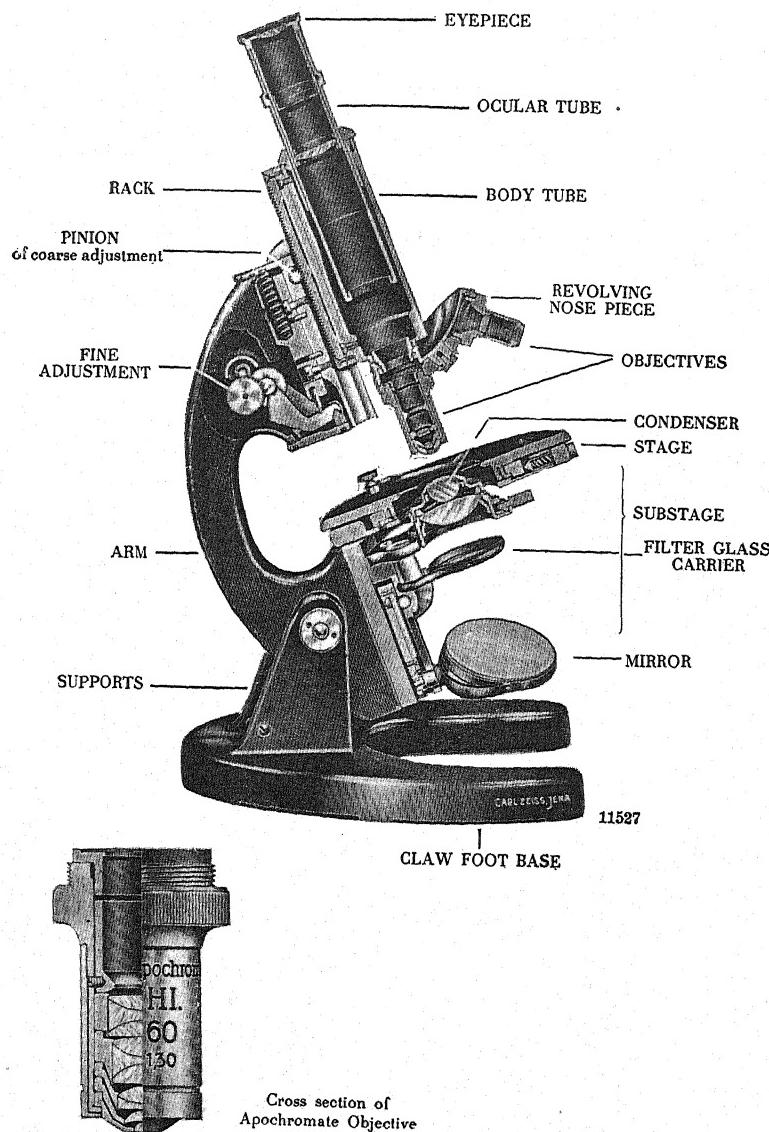


FIG. 10. Cross section of a compound microscope (Zeiss) with the various parts named. At the lower left is shown an apochromatic objective in partial cross section. Although ten separate lenses are required in its construction, they all function as a single lens.

those portions of the microscope which function like the photographic lens to produce the enlarged image.

If the focal length of the microscope objective be so chosen that the relation between the conjugate foci of the object and image planes is as 1:10, the image formed by the objective is magnified 10 times. We can then designate such objective as a 10*x*, implying that it possesses an initial magnification of 10 diameters.

In a compound microscope the position of the image plane is always fixed by the manufacturer so that it will lie at the proper place for the eyepiece to pick it up and further magnify it. But as the latter can always be focussed, in combination with the objective, to yield a sharp image on the sensitive film, no matter where the latter may be located, it becomes necessary to establish a reference distance corresponding to the magnification one obtains when using the microscope for visual purposes. This distance is the same as that assumed as the distance of best vision for the normal eye, i.e., 10 inches. If, then, a screen is placed 10 inches from the eyepiece (specifically, the Ramsden circle, or eyepoint, where the rays cross, just outside the eyepiece), the image projected on it will correspond in magnification to that seen by the eye in visual work with the microscope. This means that if the eyepiece be a 10*x*, a 10 times enlarged image formed by the objective would be again magnified 10 times, making 10×10 or 100*x* total magnification.

Thus a microscope capable of yielding a 100*x* image at a 10-inch distance can be conceived of as the optical equivalent of a single lens of $1/10''$ focus. Considered in this manner, the relationship between ordinary photography and photomicrography, whatever might be the magnification of the microscope lenses employed, is easily recognized.

A glance at the microscope depicted in Figure 10, however, reveals that there are other parts to the instrument, in addition to those required to form the image. From the optical standpoint the principal one of these is the condenser, located beneath the stage. Its function is properly to illuminate the object on the stage when the latter is being viewed or photographed by means of transparent illumination.

In one respect photomicrography is radically different from ordinary photography. In the latter we are dealing almost exclusively with objects which are opaque and therefore photographed by the aid of light reflected from them. Only occasionally are silhouette photographs taken, with the source of illumination located behind the object. The condition usually encountered in photomicrography is the reverse of this. Here the major part of the work is done with light

passed through the object, the latter being sufficiently thin and transparent to enable this to be done. Only in the photographing of surfaces of opaque objects is the condition similar to that of ordinary photography.

The employment of transmitted light necessitates the addition of the condenser, whose function, as will be discussed in detail later, is to condense or focus an intense beam of light onto the object.

The fact that the three optical parts of a microscope require proper alignment with each other, rigid support, and means (both coarse and fine) for adjusting the focus to secure a sharp image on the image plane, explains the need for the other mechanical parts of the instrument. Also, the object must have a stage upon which it can be located with respect to the objective. Further, it usually happens in visual work, and often in photomicrographic work, that the source of light is located somewhere away from the optic axis, so that to pick it up and project it into the condenser a mirror is required.

This, in a brief way, gives the reasons for the essential parts of a compound microscope. The actual design of the instrument may vary over wide limits, but basically in every one will be found all the features necessary for general work. Higher-priced outfits have many additional features. These may include circular rotating stages, mechanical stages for moving the object (i.e., the glass slide upon which the latter is mounted), more complete substages apparatus with decentering diaphragm, binocular body tubes for visual work, etc.

The Optical System of the Compound Microscope

(a) Objectives

The most critical piece of optical equipment of the compound microscope is unquestionably the objective. Especially is this so in photomicrography, where the demands made upon it are even more severe than with visual work. Imperfections in the image formed by the objective, due to the latter's falling short of perfection, are naturally aggravated by further magnification with the eyepiece. Moreover, the initial magnification of an objective intended for high-power work may amount to well over 100 \times , though it is unusual to employ eyepieces higher than 10 \times to 20 \times .

Whatever the magnification of the eyepiece it cannot possibly bring out any resolution of detail not already present in the image produced by the objective. All it can do is to make such detail as is present more easily seen by the eye.

The perfection of the objective is therefore of prime importance to the microscopist and every manufacturer of microscopes endeavors to produce the utmost possible. As regards the degree to which aberrations have been eliminated, objectives can all be grouped into three classes. These are designated Achromats, Fluorites (or sometimes Semi-apochromats), and Apochromats.

Achromatic objectives are corrected for spherical aberration for one color (designated the preferred color) of the spectrum, usually the yellow green, as this is near the central portion of the visible band. The residual imperfections due to spherical aberrations in the regions on each side of the yellow green are thus minimized, although they become greater in proportion as the color is removed from the preferred color.

In addition to spherical aberration correction for one color, achromatic objectives are also corrected for chromatic aberration by superimposing two spectral color regions so that they also come to a focus at the same point. Doing this brings all the other color regions close together, and a very good image results, especially for visual purposes.

Apochromatic objectives are still more perfectly corrected. This is possible through the use of the mineral fluorite, which possesses characteristics not obtainable in any known glass. Spherical aberration is corrected for two regions in the spectrum instead of one only, as in achromatic objectives, and three colors have been superimposed in correcting for chromatic aberration, thus making such lenses almost ideal. One limitation only is present in apochromatic lenses: they require specially corrected eyepieces known as compensating oculars to be used in combination with them, because a part of the correction is accomplished in the eyepiece.

The third series of objectives, known as Fluorites or Semi-apochromats, are intermediate between the other two, being much superior to achromatic lenses but not quite the equal of apochromats. Like the latter, they require compensating eyepieces in order to assure their best performance.

For visual work, high-quality achromatic objectives are very satisfactory. As a matter of fact, only an expert microscopist would be likely to detect the difference in the images produced by achromatic and apochromatic objectives, unless they were seen simultaneously with the aid of a comparison eyepiece.* As to photomicrography

* The comparison eyepiece is a device which can be fitted over two microscopes, side by side, the images from them being brought together into one eye lens in such manner that one-half of the field is the view through one microscope while the other half of the field is that through the other microscope.

with achromatic objectives, however, their performance differs markedly from that of apochromats. Only when employing a green filter which passes the particular spectral region for which the lens usually is best corrected does an achromat yield an image approximating the results obtainable with an apochromat. On the other hand, for many purposes a fluorite objective is practically the equal of an apochromat, at least in the center of the field.

(b) Eyepieces

Eyepieces ordinarily consist of more than one lens component, usually two, the lower of which is known as the field lens, the top one being designated the eye lens. They can be divided into two general groups — Ramsden, or positive combinations, and Huygenian, or negative combinations. The latter are far more commonly used than the positive type. It may seem a contradiction of the statement previously made that a negative lens is not capable of yielding a real image, that a negative combination can be used as an eyepiece to magnify further the image formed by the objective. There is, however, actually no contradiction involved.

It will be found that a Ramsden, or positive, eyepiece can be used as a hand magnifier, while a Huygenian cannot. On the other hand, a negative lens can take an image already formed by a positive lens and modify its size.

In Huygenian eyepieces, both components are positive elements; it is only in the way in which they are related to each other that the combined effect is that of a negative lens. Actually, in the formation of the ultimate microscopical image, either visual or photographic, the two components function separately to produce the desired result.

A more important classification of eyepieces from a practical working standpoint is the division into ordinary and compensating eyepieces. Both of these can be of either the Huygenian or Ramsden type, although the former is far more common in ordinary and compensating oculars; the Ramsden is limited largely to the high powers of the compensating type.

The primary purpose of compensating oculars is to effect a final correction in the degree of magnification of the blue and red images of apochromatic objectives, which are not of the same size. Use of compensating oculars obviates this defect so that the images coincide on the photographic plate.

An objective is capable of producing a magnified image on a photo-

graphic plate, when used without an eyepiece. Under this condition it functions exactly as if it were a short-focus camera lens, with the object distance less than the image distance. The magnification is, of course, much less than where an eyepiece is employed, being equal to

$$\frac{\text{distance from objective to plate}}{\text{optical tube length of objective}} \times \text{initial magnification of object}$$

or, expressed another way, the distance from the optical center of the objective to the plate, divided by the equivalent focus of the lens, assuming the image is formed sufficiently distant to be considered at infinity.

Hence it is common practice among some microscopists to utilize this method of obtaining lower magnifications than they can secure with objective and eyepiece combinations at their disposal. Results obtained in this manner are never ideal unless achromatic objectives, corrected for infinity, are employed with a bellows extension approximating infinity, or both are corrected for the same finite distance. Ordinary objectives of all kinds, intended to be used with eyepieces, are corrected for a definite tube length. They perform properly only under this condition. When the tube length is extended many times that for which the objective is corrected, the resulting image is inferior.

In addition to this condition apochromats are subject to a further limitation, that of a difference in the size of the blue and red images, to which reference has already been made. This can be taken care of, assuming that both the blue and red images are required to register on the plate, only through the use of properly corrected eyepieces. Of course, should color filters be employed to suppress completely all but a narrow band of color, this difference in the size of the color images is no longer a factor.

(c) The Substage Condenser

The common use of the microscope with transmitted light necessitates employing a condensing lens or system, located beneath the stage, for the purpose of concentrating a cone of light on the object, comparable to the aperture of the objective.

Usually microscopes for visual work are equipped with but one condenser, which must therefore be more or less universal in its adaptability to the entire range of objectives. When used with an oil-immersion objective having an aperture of N.A. 1.30, to secure the

utmost result of which the lens is capable, the condenser must also possess a possible aperture of 1.30 N.A. To provide for this variation, an iris diaphragm becomes essential, and is therefore always furnished as a part of the substage.

Condensers are available in simple, uncorrected types, known as Abbe 2-lens and 3-lens condensers. The latter gives a maximum aperture of N.A. 1.40, while the former is limited to about N.A. 1.20. In either case the top of the condenser must be in oiled contact with the bottom of the slide in order to secure the maximum N.A. If not oiled, the numerical aperture of any condenser is limited to N.A. 1.00. Reducing the size of the diaphragm opening effects a reduction in the aperture of the condenser to adjust it to the desired N.A.

In addition to uncorrected condensers, others are available, in which spherical or chromatic aberrations (or both) are corrected, just as in objectives. For photomicrographic work it is highly desirable that a condenser corrected at least for spherical aberrations be available. Such are known as *Aplanatic* condensers. The reason for this will become apparent as we discuss the theory of illumination pertaining to transparent objects.

In addition to the ordinary high-apertured substage condenser, a complete outfit for photomicrography in the low magnification ranges must include low-power simple lens condensers, as well; the extremely short focus of the standard condenser is capable of illuminating but a minute portion of the area photographed at low magnification.

The Theoretical Aspects of Resolution

Having considered the design and purpose of the optical parts of the microscope, we are now in a position to examine some of the factors related to the formation of the best image of which any given combination is theoretically capable. As already pointed out, there is a limit to the resolution obtainable with any objective and while there is no actual limit to the amount of magnification possible, all beyond a certain limiting value gives no increase in what can actually be seen.

After all, the only purpose in producing a magnified image of a minute object is to reveal additional information as to its structure. When we reach the point where enlargement provides no additional revelation of structure, further amplification becomes worthless.

We have also seen that the ultimate possibilities in the way of resolution are controlled, or rather limited, by the objective. The eyepiece

can add nothing to the image formed by the objective; it can only enlarge it so that detail already present can be seen by the eye. In photomicrography still another factor contributes to the ultimate enlargement. This is the bellows length. Increased magnification secured by the use of a longer bellows is similar in effect to the employment of a higher-power eyepiece. It cannot succeed in bringing out detail that is not in the image formed by the objective.

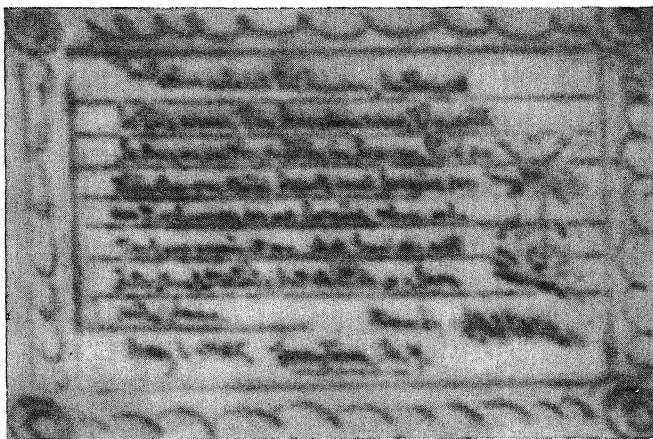


FIG. 11. Engraving on Glass, 1000 \times
Showing empty magnification resulting from use of an objective of only .30 N.A.

Figures 11 and 12 illustrate the difference between empty magnification and effective magnification. The former is a photomicrograph in which the enlargement has been pushed far beyond the point where useful magnification ends. The object is one which lends itself admirably to this particular demonstration. It is a line engraving done with a diamond on glass, the work of Mr. Alfred McEwen of Tarrytown, N. Y.*

But the lines are not sharp and clear and the writing cannot be read,

* The method of making these minute writings and the machine used to produce them are described in an article by Mr. McEwen in the *Scientific American* for June, 1923. These writings undoubtedly represent the finest handiwork of man. The particular one shown here contains the Lord's Prayer written in a space only 35 microns by 50 microns. Only to those accustomed to thinking in terms of minute dimensions do these figures mean anything. It conveys a better idea to the lay mind to state it in terms of sizes with which we are familiar. In one square inch this same writing could be duplicated nearly 370,000 times! This means that the entire Bible, both Old and New Testaments, could be written at the same size within the area of one square inch *at least 30 times over!*

although the magnification of 1000 diameters should be ample. It is obvious that increasing the magnification to double that employed would not help in deciphering the writing. In Figure 12 we see the same object at a magnification of 1500 \times . Here the enlargement has not been secured by high eyepiece and long bellows length alone but

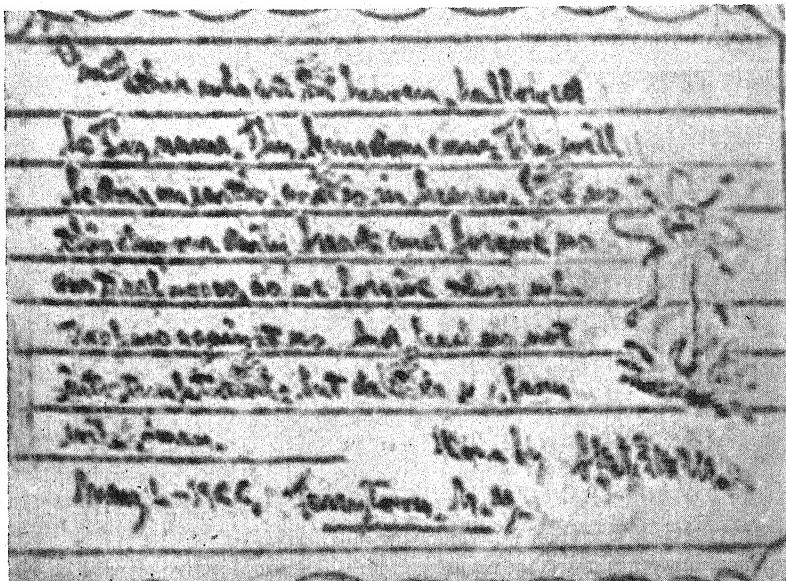


FIG. 12. Engraving on Glass, 1500 \times . Taken with an oil-immersion objective of 1.05 N.A.

The fine lines produced by the diamond point are invisible as written. They have been made visible by filling them in with a soluble dye. Hence it is the dye retained in the lines (and some still on the surface) that is actually seen. This results in a somewhat smearable effect, although the detail is so perfect that, on the original print, the grain of the dye can be easily seen in some of the lines.

by the use of an objective capable of yielding more detail as well as higher magnification in its primary image.

From this it is evident that there are certain fundamental laws relating to the formation of ideal images, with which the photomicrographer should be familiar. Inasmuch as the eyepiece and bellows length enter the problem only as amplifiers of the original objective image, it is clear that we can consider them as an integral part of the entire magnifying unit, and deal only in terms of the ultimate size of the final projected image for any given objective.

The presence of a "circle of confusion" in place of an absolute

point, in the image of a luminous point, and the relationship of the numerical aperture of an objective to the size of this circle of confusion, have already been mentioned. It has also been stated that the limit of useful magnification has been set at approximately 1000 times the numerical aperture of the system. We are now ready to examine the way in which this figure has been determined.

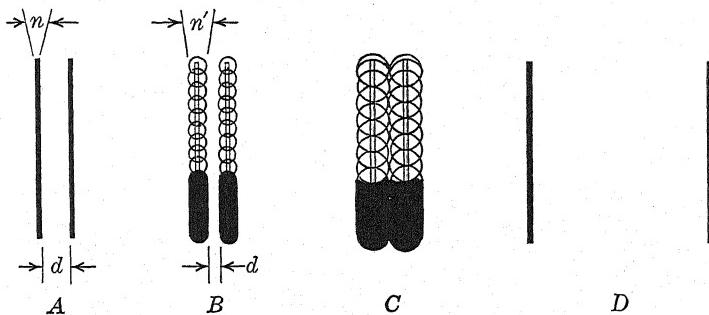


FIG. 13. Effect of size of circle of confusion on resolution.

Let us for the moment ignore any part the human eye may play and consider only the effects present in the image as formed by a lens.

In Figure 13*A* we have two lines of a width n separated by the distance d . These lines can be conceived of as a continuous series of points of a diameter n . Therefore, when we photograph these lines, every point in them will be reproduced, not as having a width n but as circles of confusion having a diameter of n^1 , as in Figure 13*B*, and the thickness of the lines would appear in the photograph as in the lower half of *B*. Should the diameter of n^1 be sufficient for the circles to overlap, as in Figure 13*C*, the photograph would no longer show the lines as two separate narrow lines, but as one broad line as in the lower portion of *C*. Thus as dimension d is progressively diminished, it is obvious that to reveal the lines as separate, the size of the circle of confusion must become increasingly smaller, or, in other words, the aperture of the lens employed must be proportionately increased.

The circle of confusion is formed as a result of diffraction, i.e., the scattering of light which occurs when light rays pass through an aperture. This scattering varies with the wave length of the light (as well as the angular aperture), and hence this factor must enter into any formula which expresses the amount of diffraction, the size of the circle of confusion, or, what is more practical from the microscopist's viewpoint, the degree of resolution of which any lens is capable.

The trigonometrical formulation of the resolution characteristics of a lens in practical terms is somewhat involved, and is not of direct interest in microscopical work, but the final formula as derived is relatively simple and should be memorized and understood from the standpoint of its effects in the production of ideal micrographs. It is:

$$R = \frac{\lambda}{2 \text{N.A.}}$$

in which R is resolution, in terms of the minimum distance (in microns) between two points in an object which will just be revealed as separate points in the image. λ is the wave length (in microns) and N.A. the numerical aperture of the system. To find the number of lines per inch which will be resolved, the value in microns derived from this formula is divided into 25,400.

It is apparent that the question of magnification does not enter this formula at all. This is because magnification must take into account an entirely different condition — that is, making the final image sufficiently large to allow the resolution of which an objective is capable, to be seen by the human eye. Just as actual detail exists in the object itself which is much too fine to be seen by the unaided eye, so can it exist in a magnified image of the object which is still not sufficiently large to be seen.

Thus in a photomicrograph it becomes the function of the eyepiece and bellows length further to enlarge the original objective image to a point where the eye can appreciate it at the distance of best normal vision, i.e., 10 inches from the eye.

The fact that the function of the eye plays a part in the final magnified image — whether the visual image observed on looking into the eyepiece, or the photographic image recorded on sensitized films — means that we must approach this part of the problem from the standpoint of the eye before we can associate resolution with magnification.

In Figure 13D are two lines, separated a distance of approximately one inch. With the eye placed 10 inches from the lines the sine of angle which they subtend will be $1/10$. The angle corresponding to $.10$ is $5\frac{1}{2}$ degrees. The eye observes the lines with this spacing as very widely separated. But if by some means we could move the lines closer together we would reach a point where the space between them would be just visible; any further movement would cause them to blend into one apparent line.* The distance between them under

* Since the eye lens obeys the same laws as other lenses we must expect diffraction to be present, and thus a circle of confusion exists in the image in the eye, just as in

this condition would be found to vary with different individuals; some can see them as two separate lines much closer than others can. With most individuals, when the separation reaches $1/100$ of an inch, they begin to emerge; very few can detect lines separated only $1/200$ of an inch and held 10 inches from the eye. It is important to retain the 10-inch dimension as a reference standard, since the movement of the lines to a point 5 inches from the eye would be equivalent to doubling the angle, which in turn is as if the lines were moved twice as far apart. Only very near-sighted individuals can focus sharply on an object 5 inches away, and so this is not a normal condition.

For all practical purposes, the $1/100$ inch (250 microns) separation can be assumed as a fair value to use in determining the resolution and magnification necessary under working conditions. Though we started out with a consideration of the separation of two lines, it must be understood that the same condition obtains in the case of two separate points, and hence we can talk in terms of either lines or points in an object (or its image) indiscriminately.

Suppose we have an object, such as a diatom, which has line markings on it that are spaced $1/10,000$ of an inch (2.5 microns) apart. How much must it be magnified in order that the lines in the image will be seen by the eye and the structure of the object itself be revealed?

The first part of the computation is easy; the $1/10,000$ " must be magnified until it appears $1/100$ ", which would be 100 times. Any less than this amount would not be sufficient to allow the eye to discern the line markings.

But we have already seen that mere magnification is not enough; the objective must have the ability to resolve the structure at this magnification. This must be determined by reference to the resolution formula

$$R = \frac{\lambda}{2N.A.}$$

a camera. In the eye, however, the circle of confusion is not the limiting factor. We can conceive of the grain of a sensitive film, or the screen upon which an image is projected, as substantially continuous in area, but this is not the case in the eye. The image is picked up in the retina by the rods and cones, and hence is analogous to the image produced by an electrotype printing. In order to be observed as separate lines, the image of the lines in the eye must fall upon different rows of cones in the fovea. When the lines are so close together, regardless of the circle of confusion present, that the images of the lines fall on the same retinal cones, the eye can no longer separate them.

which we will transpose to

$$\text{N.A.} = \frac{\lambda}{2R}$$

λ can be assumed to be a green light with a value of .500, which is substantially the approximate center of white light. R will be $250/100$ microns if the magnification is set at $100\times$ and the allowable circle of confusion is 250 microns (i.e., $1/100$ inch). Inserting these values in the formula we have:

$$\text{The required N.A.} = \frac{.500}{2 \times 250/100} \quad \text{or N.A.} = .10.$$

This numerical aperture is $1/1000$ of the magnification, and hence it is common practice to state that the limit of useful magnification with ordinary white light (central at approximately .5 microns wave length) is 1000 times the N.A. of the objective. Assuming the highest possible correction in the objective and all other factors approximating ideal conditions, photomicrographs taken at a magnification of 1000 times the numerical aperture of the objective will be wonderfully crisp and sharp in detail. They should stand examination under an ordinary reading glass without appearing to lose sharpness. This suggests that often, in actual practice, the magnification can be pushed considerably beyond the theoretical limit, with even an apparent gain in detail. The reason for this is usually overlooked. It lies in the nature of the image as a whole which is seen by the eye. The total angle of vision of the eye is very large but acuity of vision is limited to one particular minute spot in the eye, known as the *fovea*. When we wish to examine anything critically it must be focussed exactly on the fovea. Surrounding the fovea is a larger area, the *macula*, which provides less perfect vision although still of a high order. Beyond this the quality of the image drops off considerably. As one reads the letters on a printed page, it is done by a process of scanning, each letter in turn being passed rapidly over the fovea. But it is much easier to read large print than small because the imperfect image of the larger type can be partially interpreted by the retina before reaching the fovea, and the concentration required becomes materially less.

Examination of a photograph follows the same law. The eye must scan every portion of it (subconsciously, of course) to pick up all the detail revealed. Therefore, if we enlarge the image until a large portion of its interpretation can be effected with the area of the retina ly-

ing beyond the fovea, fatigue is materially lessened and the pleasurable sensation which results therefrom is heightened. In other words, we can *see* the picture with less exertion. At the same time, concentration on any particular detail will reveal that there is less sharpness than at the enlargement where theoretical "empty magnification" is not present. Examples of magnifications far in excess of 1000 times the numerical aperture of the objective employed will be given later.

When, for any reason, it becomes desirable to check the magnification, numerical aperture, wave length, and desired resolution against each other in connection with the resultant photomicrograph, it is apparent that the resolution formula need only be transposed to put the factor sought on one side of the equation and all the other factors on the other side.

In the basic formula $R = \frac{\lambda}{2N.A.}$, we must break down R into two

separate factors, the diameter of the circle of confusion (designated d) and the magnification (M). As long as we are satisfied with a circle of confusion of $1/100$ inch (250 microns), d would always be 250; but it is possible under some conditions that greater sharpness might be desired. Then d might be any value between 250 and a low limit of 75 microns, the latter covering the extreme resolution of which the human eye is capable. R in the formula will then be replaced by d/M . The following are the possible arrangements of the formula, and the numerical values derived on the basis of the problem already given.

$$(1) R = \frac{\lambda}{2N.A.} \text{ when the resolution is required,}$$

$$\text{i.e., } R = \frac{d}{M} = \frac{.5}{2 \times .10} = 2.5\mu \left(\frac{250}{100} \right)$$

$$(2) N.A. = \frac{\lambda}{2 \times d/M} \text{ when the numerical aperture is required;}$$

$$\text{i.e., } N.A. = \frac{.5}{2 \times 250/100} = .10N.A.$$

$$(3) d = \frac{\lambda M}{2N.A.} \text{ when the diameter of the circle of confusion is required.}$$

$$\text{i.e., } d = \frac{.5 \times 100}{2 \times .10} = 250 \mu$$

(4) $M = \frac{2 \text{N.A.} \times d}{\lambda}$ when the magnification is required.

$$\text{i.e., } M = \frac{2 \times .10 \times 250}{.5} = 100 \text{ diameters}$$

(5) $\lambda = \frac{2 \text{N.A.} \times d}{M}$ when the wave length is required.

$$\text{i.e., } \lambda = \frac{2 \times .10 \times 250}{100} = .5 \mu$$

Thus it is evident that by memorizing the simple basic formula for resolution, one can easily ascertain any factor necessary to meet a given condition.

Illumination

The important role played by illumination in the production of ideal photomicrographs of transparent preparations is largely ignored by the average microscopist. This, rather than the quality of the optical system, is mainly responsible for the mediocre results so often achieved. Even the best of objectives can perform but poorly when the illumination is not correct.

This is equivalent to saying that the performance of the objective can be modified or limited by some other part of the optical system, which is true. In the formation of the image the objective can utilize only rays which reach it from the object, and hence if such rays are not sufficient to produce an image of the quality the objective is capable of forming, the image will fall short of ideal by the amount of the deficiency.

It is the function of the condenser to provide adequate and ideal illumination of the object. This has nothing to do with the intensity of the light; it concerns only the angular relation of the cone of light formed by the condenser, to the aperture of the objective.

Early recognition of this important relationship between the objective, condenser, and illumination source, on the part of some of our pioneer microscopists, resulted in the working out of the method of producing the best results and the coining of a term by which the

method could be designated. This term is *critical illumination*. Just what is critical illumination and how is it related to image formation?

Critical Illumination

The ordinary substage condenser is, in effect, a very short-focus lens combination, quite similar to an objective, but facing in the opposite direction. Rays from a distant light source are thus brought to a focus by the condenser, to form an image of the light source. The condenser position is then so fixed (by adjustable focussing means) that the image plane of the light lies directly upon the plane of the object, upon which the objective is focussed. This condition is shown in Figure 14. The maximum angle of the cone of light forming the

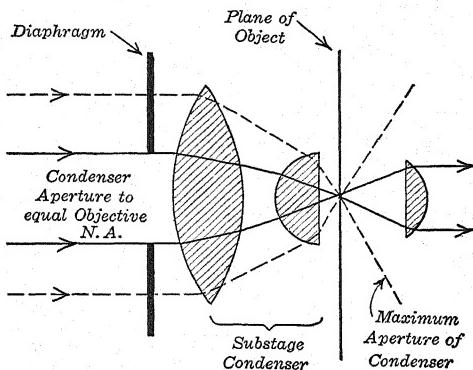


FIG. 14. Principle of critical illumination, where the image of the light source is formed in the plane of the object

condenser image is shown by the outer dotted lines, assuming the diaphragm to be wide open. By closing the diaphragm to the point where the resultant cone of light from the condenser is just equal to the cone which can be picked up by the objective, theoretically perfect illumination is provided. It is to this particular arrangement that the name *critical illumination* (or *critical light*) is given.

It is only under this condition that the full resolution characteristics of an objective can be realized. The part played by the condenser is reflected in the resolution formula which we have already considered. To give it in its fullest form it should actually be written:

$$R = \frac{\lambda}{2 \left(\text{objective N.A.} + \text{condenser N.A.} \right)}$$

This is because the effective numerical aperture system as a whole is the *average* of that of the objective and condenser. When the best possible condition obtains, the N.A. of the condenser is equal to that of the objective and the formula reduces to:

$$R = \frac{\lambda}{2 \text{N.A.}}$$

The poorest condition is represented by no condenser at all. For instance, taking a concrete example, where an objective with an N.A. of .50 is used without a condenser (i.e., the N.A. of the latter is substantially 0). Then we have

$$R = \frac{\lambda}{2 \times \left(\frac{.50 + 0}{2} \right)} \text{ which becomes: } R = \frac{\lambda}{.50}$$

In other words, without a condenser the resolution is just one-half or

$$R = \frac{\lambda}{\text{N.A.}}$$

It might appear from this that increased resolution would result from employing a higher aperture in the condenser than that available in the objective, but this is not possible, because of the introduction of a new complication. This will become evident when we discuss dark-field illumination. The N.A. of the condenser must never *exceed* that of the objective.

The theoretical aspect of critical illumination is quite involved. There are two schools of thought, each of which attempts to explain the underlying principles of resolution on different premises. It is not necessary that the practical microscopist be familiar with the fine points of the controversy in order to do the best work. It is, however, desirable to be able to appreciate the two conditions which obtain, under slightly varying arrangements of the light condensing systems, now supplied with photomicrographic equipments. Both are of value under certain conditions, so that a knowledge of when to employ one method and when the other is decidedly worth while. To make the situation clear, it will simplify matters to approach the explanation from the historical side.

As originally conceived, critical illumination was obtained by the

simple expedient of focussing the image of the luminant directly, without the interposition of a luminant condenser. The condition was as illustrated in Figure 15*A*. In the early days of microscopy, suitable light sources, such as we have now, were not available, and so difficulties were present both in the securing of a light that would cover the entire field and also in obtaining an even illumination in such portions of the field as were covered. Figure 15*B* illustrates what the appearance would be on looking through the microscope, with a

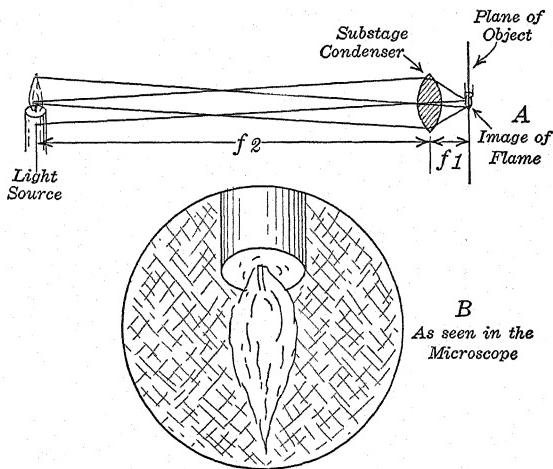


FIG. 15. The most primitive conception of critical illumination

candle as the light source, under the condition of critical light. The most frequently used artificial light source was the flame of a kerosene lamp turned edgewise, not radically different in general aspect from a candle.

There are several ways to obtain a greater coverage of the field. The simplest is to move the light closer to the microscope, but because of the extremely short focus of the Abbe condenser even this could not cause the image of the light source to cover the entire field when low-power objectives were in use.

A better substitute was soon found in the employment of a so-called "bull's-eye condenser," located in front of the light source a distance equal to the focal length of the condenser. The rays would emerge from the condenser parallel and would thus represent the condition of a much larger source of illumination, reaching the substage condenser as though coming from infinity, as shown in Figure 16*A*. The

focus (f) of the substage condenser would be a little shorter than f_1 , in Figure 15A, under this condition; hence the condenser must be moved a little closer to the object to form an image of the light source, but when properly focussed, the image would be materially increased in size, as in Figure 16B.

The weakness in this method of securing critical illumination lay in the unevenness of the lighting over the entire field. This is inevi-

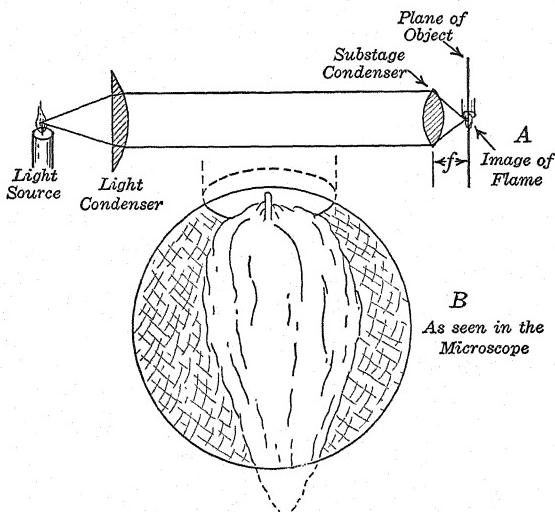


FIG. 16. Improvement in critical illumination secured by employing a light condenser positioned to yield parallel rays

table with such lighting, as each portion of the field is illuminated by the rays from a corresponding point in the light source. Whatever variations might be present in different parts of the flame would be evident in the image of that flame used to illuminate the field of view. To obviate this condition it was often suggested that the position of the substage condenser be altered to throw the flame image slightly out of focus. But immediately this is done, the lighting is no longer critical.

With amateur microscopists continually striving to secure uniform illumination over the entire field of view by juggling the relative positions of the substage and bull's-eye condensers, it was inevitable that a slight change in the position of the latter would be found to work wonders in providing an evenly illuminated field of view. This change consisted in moving the bull's-eye condenser a little nearer the micro-

scope than the position represented by its focal length, until the rays from it, instead of being parallel, were slightly converging, coming to a focus on the diaphragm of the substage condenser. The path of rays under this condition is as shown in Figure 17. With the substage condenser in the position to provide critical light, it is found that *the bull's-eye condenser has become the apparent light source*; the flame is no longer seen. This method of obtaining critical light was known as "imaging the light condenser" in the plane of the object. It was not originally conceived of as being any different in principle from critical illumination obtained by imaging the light source with parallel

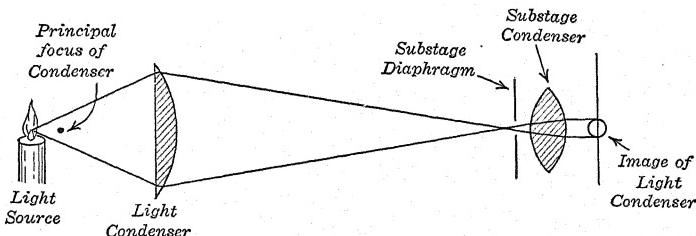


FIG. 17. Critical illumination secured by imaging the light source in the plane of the substage diaphragm. Sometimes called Köhler illumination

rays from a bull's-eye condenser. Or, to put it in another way, the bull's-eye condenser, when imaged in the plane of the object, was considered analogous to a disc of ground or opal glass, situated in the same position as the condenser and illuminated evenly all over by the light source in back of it; this ground glass provided both greater area and more even distribution of the light than was possible with the light source alone.

While in effect this is substantially (although not altogether) true, it remained for A. Köhler * to demonstrate mathematically that illumination obtained in this manner is theoretically different in principle from that resulting from imaging the light source itself in the plane of the object. For this reason there is a tendency to designate the condition where the condenser is imaged in the plane of the object as the "Köhler method," limiting the name "critical illumination" to imaging the light source itself in the object plane.[†]

* A. Köhler, "Zeitschrift für wissenschaftliche Mikroskopie," volume 10, pages 433-440 (1893).

[†] English microscopists do not take kindly to this differentiation, especially as applied to medium- and high-power illumination, inasmuch as Köhler made no claim to having actually originated the method and it was in common use long before he published his analysis and practical application of it. This is a cause of some confusion if one derives his information from publications originating in England.

If this is done solely on the basis of having a means of differentiation between the two systems of illumination, it is justifiable, but if there be an implied idea that Köhler illumination is *not* critical, a false impression results, for the lighting is, or can be, equally "critical" whichever method is used. The true test of critical illumination is that all illuminating rays are uniformly disposed about the optic axis and of sufficient aperture entirely (and just) to fill the back lens of the objective.

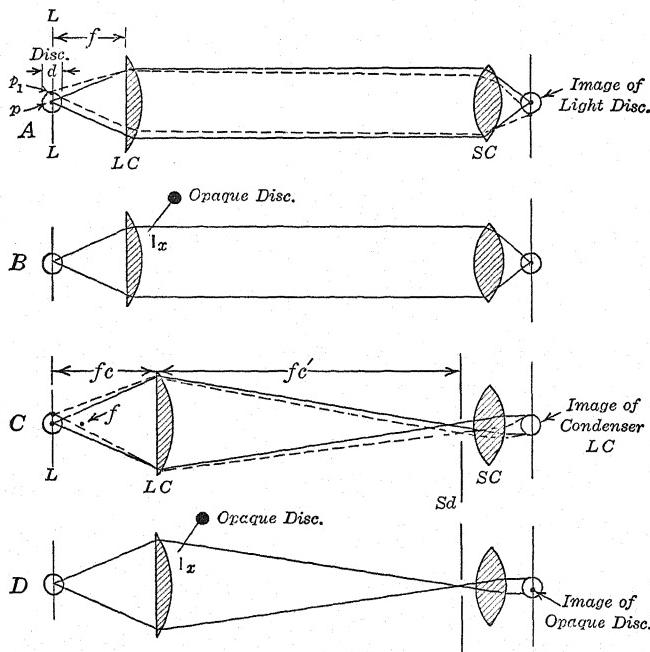


FIG. 18. Comparison of the two methods of obtaining critical illumination for medium and high magnifications

It is not our purpose, at this time, to analyze the theoretical aspects of the two systems of illumination in their relation to image formation through interference maxima, but it is important that the fundamental differences between them be understood by the practical photomicrographer, that he may know which to use under varying operating conditions. To this end, let us follow out the course of some of the rays from the light source to the object plane, under each system.

In Figure 18A we have two pairs of limiting rays plotted, under the setup usually known as critical illumination, analogous to Figure 16A. The light source we will assume as an evenly illuminated disc

of a diameter d located in the plane $L - L$. The disc is shown turned around so that we may conceive its surface aspect; actually, it is flat and lying in a plane at right angles to the eye. The same applies to the illuminated area as well. LC is the lamp condenser, with a focus = f , located its focal distance from the plane of the light source. All rays from any one point (as at p) on the disc, after passing through the condenser, emerge parallel. Upon reaching the substage condenser (SC) they are brought to a focus to form an image of the point from which they originated in the focal plane of LC . As already explained, the position of SC is so regulated that its focal plane coincides with the plane of the object. Every other point on the light source disc — e.g., p_1 — forms its corresponding part of the image; the latter, however, is inverted. Should p_1 in the light source be an opaque spot, a corresponding dark area would appear in the image and be observed in the field of the microscope. Also, if we reduce the diameter of the light disc — as, for example, by means of an iris diaphragm — the image will also be reduced. The actual size of the light image in the object plane can be determined on the following basis:

$$\text{image of light : size of light} :: \text{focus of } SC : \text{focus of } LC.$$

As the focus of a substage condenser of N.A. 1.40 is extremely short, the diameter of the illuminated area is small. This means that the entire field of low-power objectives will not be covered unless means are available to increase the size of the illuminating source.

With this setup, if a small black disc were placed in front of condenser LC , as at x in Figure 18B, it would have no effect on the image of the illuminating disc (except for a slight reduction in the total amount of light), for the rest of the condenser area would still function to form the image.

Consider now the second case, illustrated in Figure 18C. The only change in the arrangement is that the light condenser, LC , has been moved a little farther from the light source than its principal focus (f) until its distal focus, no longer at infinity, lies in the plane of the substage diaphragm, sd . The conjugate foci are now fc and fc' , respectively. The substage diaphragm is located near the back focal plane of the substage condenser. Rays from a point in the light source passing through condenser LC , after forming an image of the point in the light source, in the plane sd , cross and continue on through condenser SC , emerging nearly parallel, to form a disc of light in the plane of the object. Thus, even if the light source were not a disc of appreciable

diameter, but a theoretical point, a relatively large disc of illumination would be projected on the plane of the object.

As the diameter of the light source is increased, each point on its surface projects its disc of illumination on the object plane, all superimposed on each other. Irregularities in the intensity of the light at different points, or completely opaque areas within it, would make no difference on the uniformity of the illuminated area. On the other hand, should an opaque disc be placed in front of condenser LC , as at x in Figure 18D, it would show as a black area in the field of the microscope, thus proving that the illuminated disc formed in the plane of the object is an image of the condenser, LC , which is evenly illuminated by the light source, whatever the nature of the latter may be. The diameter of the illuminated area is proportionate to the diameter of condenser LC approximately as the focus of the substage condenser is to the length of the conjugate focus fc' .* Thus we can reduce the diameter of the field by placing an iris diaphragm in front of the light condenser, or we can increase it, within limits, by the use of a larger-diameter light condenser.

In either of these systems, as the focal length of the substage condenser is a factor in determining the size of the area of illumination on the object plane, it is evident that the use of a substage condenser of longer focal length will also serve to increase the diameter of the field illuminated, although at the expense of a reduction in its ultimate numerical aperture.

If the photomicrographer can master the practical application of these two systems so as to be able to employ either at will, he need not worry over the fine points of the controversy which has been waged over the relative merits of each, from a theoretical standpoint. This has to do with the question of the value of coherent light in the formation of interference maxima, upon which resolution is postulated to depend.†

* The absolute relationship is true only under the theoretical condition where the plane of the substage diaphragm lies in the exact rear principal focus of the condenser, so that rays from the latter emerge parallel. Because of mechanical design limitations, this is usually not the case, the diaphragm being located farther from the condenser. Should the distance be equal to $2f$, the equation becomes:

$$\frac{\text{diameter of illuminated area}}{\text{diameter of light condenser}} = \frac{zf \text{ of substage condenser}}{\text{conjugate focus } fc' \text{ of } LC}$$

This is more nearly the average condition, but it does not affect the general rule as to the control of the field aperture by means of the diaphragm in front of the light condenser.

† It is axiomatic that only coherent light can produce interference phenomena,

In practically all authoritative works where these two methods of illumination are discussed, the latter, usually referred to as the Köhler method, is described as distinguished by "imaging the light source in the plane of the back lens of the objective," instead of in the plane of the object, as in so-called "critical illumination." This method of identifying it, while technically correct, is not very satisfactory from a practical standpoint, whereas it is easily comprehended by the phrase "imaging the light condenser in the plane of the object."

Neither of the systems of lighting described is satisfactory for use with low magnifications where it is necessary to cover a large field. From the practical photomicrographer's standpoint, Köhler's greatest

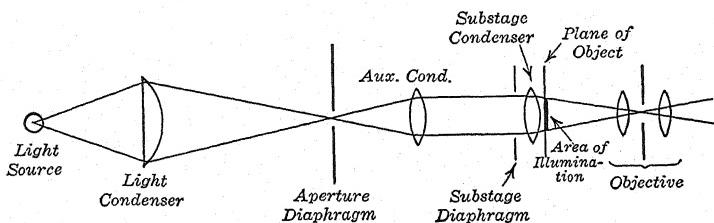


FIG. 19. Köhler critical illumination for low magnifications

contribution to the science of critical illumination is his method of covering a large field uniformly, thus meeting a need not present in the early days when microscopes were used almost solely for visual purposes.

The setup is as shown in Figure 19. For best performance all the optics are different from those in use in the medium- and high-power

through change of phase relationships. The theory propounded by Abbe, that resolution depends upon and is proportional to the number of interference maxima formed by the angular aperture of the cone of light picked up by the objective, has been endorsed by advocates of critical lighting where the light source is imaged in the plane of the object. On the other hand, it is objected that coherent light does not result from this method; that only the imaging of the light condenser in the plane of the object can provide coherent light. Thus the latter method should give better resolution, but this cannot be demonstrated. The subject is highly speculative. For instance, it can be argued that one must specify what particular rays from the light source must be coherent, in order to form the interference maxima in the image. If the light over the entire area is involved, it is correct that coherency can result only with the so-called Köhler illumination where light originating in one point covers the entire field. But it can be argued that superimposed upon the coherent light from any one point is other light, coming from an infinite number of other points, each coherent with itself, but not with each other.

If, on the other hand, it is argued that it is only the rays falling on an individual point in the object that must be coherent, such a condition can be obtained only by imaging the light source itself on the plane of the object. Thus while the light falling

arrangement. Condenser *LC* can be the same but is preferably of shorter focus. In actual practice this is often accomplished by the use of an auxiliary positive lens placed in front of the regular lamp condenser. Condenser *LC* is focussed upon an iris diaphragm which serves as an aperture stop. From here, though the rays could continue directly to the substage condenser *SC*, in actual practice it is found advantageous to insert an auxiliary condenser (aux. cond.) in the system to parallel the rays entering the substage condenser. This latter is a low-power single-lens (often called a spectacle-lens) condenser, located directly beneath the stage. Its focal length is such that when in this position, the rays from it come to a focus in the plane of the diaphragm of the low-power photomicrographic lens used with it. Whenever the latter is changed to one of another power, a corresponding change is made in the substage condenser; i.e., a battery of five objectives requires a corresponding set of five condensers.

With this system large areas, up to considerably over an inch in diameter, can be evenly illuminated. In effect, this method is analogous to that employed in the stereopticon, where it is necessary to illuminate an area approximately four inches in diameter.

When the three variant methods of illumination with transmitted light are compared with each other, certain similarities and dissimilarities are evident. Let us analyze these, from the standpoint of operation.

First, the only differences in the setup between the two systems for high-power work lie in the location of the light condenser, which is nearer the light source in one case than the other, and in the position of the field diaphragm, which must be directly in front of the light source in one case and directly in front of the light condenser in the other. The apparatus is identical in either case. The substage diaphragm controls the aperture in both cases.

on each point in the object would be coherent with itself, it would not be coherent with respect to any other point in the object.

In view of the fact that photomicrographically both methods appear to yield equivalent results, it seems possible that both premises are wrong; that the true answer lies somewhere else. It is the author's personal belief that the whole problem can be solved and the Abbe theory of resolution retained on the basis that it is not necessary to consider the primary source of illumination at all, so far as coherency is concerned. The rays which form interference maxima and which must always be coherent are those proceeding from the object itself, which may be considered as merely excited by an external source. This view need not conflict in any way with the fact that the exciting rays must have an aperture sufficient to fill the back lens of the objective. There is a very simple explanation for this.

In the second place, a point that is often overlooked is that critical lighting, within the original understanding of the term (when no lamp condenser was employed), can be secured with the lamp condenser located anywhere *between* the two limiting conditions. In other words, these two methods of illumination merge imperceptibly into each other, without any positive line of demarcation. It is impossible to detect any difference in resolution, or in the character of the image, for any intermediate position of the lamp condenser.

In the technical aspect of the two systems, there is the difference in the position where the image of the light source is located; in one it is situated in the back conjugate focus of the objective; in the other it is near the back lens of the objective. From a practical standpoint, however, this is a small difference, for while in the latter case the image of the light source is sharply delineated as one looks down the tube with the eyepiece removed, in the other case it is also seen, but is not in sharp focus.

When we come to compare low-power Köhler illumination with the other two, so far as operation characteristics are concerned, we find that radical differences exist. Chief of these is the change in the functions of the diaphragms. The substage diaphragm does not control the *aperture* of the system, but has become a *field diaphragm*. If but one additional diaphragm is employed (which is all that is actually necessary), it must be moved from its position in the front focal plane of the lamp condenser (or between that point and the front surface of the condenser) to the front conjugate focus for the low-power system. It then ceases to be the field-limiting diaphragm, becoming instead the aperture diaphragm. The condenser itself must either be changed to one of shorter focal length, or moved farther from the lamp.

Then again, the position of the image of the light source does not coincide with either of the other systems, for it is located in the optical center of the objective, i.e., in the plane of the diaphragm, when the latter is present.

It is thus seen that we have *three* separate systems, commonly referred to by only *two* designations, "critical illumination" and "Köhler illumination." All three are actually "critical" so far as performance is concerned, and therefore the nomenclature is misleading. Furthermore, the method of low-power illumination is decidedly to be credited as original with Köhler in its application to microscopy, whereas his contribution to the other method sometimes ascribed to

him was merely a scientific analysis of its operation, as distinguished from the accepted form of critical lighting. In view of all these factors, it appears logical to limit the designation "Köhler illumination" to the low-power method, describing the others under the older nomenclature, "critical illumination," with the addition of the phrase "imaging the light source," or "imaging the light condenser," as the case may be.

Discussion of the relative advantages of each of these systems from a working standpoint is reserved for Chapter 4.

It yet remains, on the basis of what has been said for the various methods of illumination, to sum up the illumination requirements essential to the best performance of a photomicrographic outfit. These are:

- (1) The illuminating rays should be symmetrically disposed about the optic axis of the microscope, and should be capable of entirely filling the area of the back lens of the objective yet not be in excess of this.
- (2) The diameter of the illuminated area should be ample to cover the entire field of view to be photographed and means should be available to circumscribe it as near as possible to this area.
- (3) The entire area illuminated should possess uniform intensity throughout.

When these conditions are met, the light is *critical*, whatever method is employed to achieve the results.

To these conditions might be added a fourth, one which is largely of convenience from a practical standpoint. This is, the light should be ample to enable careful focussing to be done on the ground glass, under any conditions of operation, and yet should not be excessive. This is the service condition which determines, more than any other, which system of illumination should be employed. It will be discussed at length in Chapter 4.

With a thorough understanding of these basic principles of photomicrography, one is qualified to begin practical work with transmitted light.

Such modifications of these principles as occur with other types of photomicrography, such as work with incident light, dark field, etc., preferably can be considered in connection with each specific line of work.

2

Modern Photomicrographic Equipment

A complete photomicrographic outfit comprises three separate units — the microscope, the camera, and the illumination equipment. While the present tendency is definitely towards universal outfits (either of the universal camera type or the large self-contained models) all manufacturers provide, in addition to their regular line of microscopes, separate units of cameras and lighting equipment. There is a wide diversity of both available. Such equipment meets the requirements of many microscopists who already possess a microscope and do not care to invest in the more elaborate complete units. Hence commercial outfits are procurable to meet any need as to simplicity, size of camera, and monetary outlay. They range from small attachable cameras, supported directly on the microscope tube and lacking any illuminating device, through large and complete equipments, to universal designs in which the microscope is a specialized model, integral with and not detachable from the rest of the apparatus.

Expense is not the only item to consider in the selection of equipment; neither is the size of picture which can be taken; both advantages and disadvantages may be found in every commercial model, large and small, simple and complex. It therefore behooves the would-be photomicrographer to become acquainted with the limitations and marked advantages of each general design. If one is familiar with one's own special problems, an intelligent choice of equipment can then be made.

For the purpose of discussing the various commercial designs available, we can group them into classes, as follows:

1. Small attached cameras.
2. Universal vertical cameras.

3. Horizontal-vertical outfits.
4. Self-contained universal models.
5. Specialized photomicrographic outfits.

Group 1 — Small Attached Cameras

In Group 1 several types of equipment are available from various manufacturers. The growing importance of 35-mm. color photographs (mounted as 2" x 2" transparencies) has resulted in practically all microscope manufacturers providing equipment for this miniature film, adapted for use with the microscope in various ways. Leitz and Zeiss were the original suppliers of camera attachments for mounting

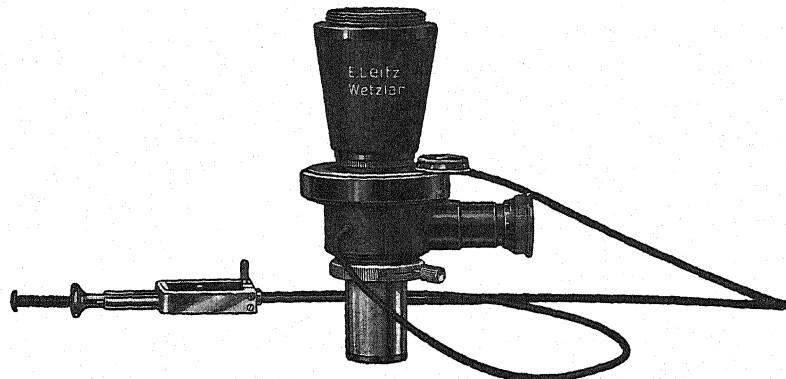


FIG. 20. Leitz Mikas (Micro-Ibso) Attachment

the Leica and Contax cameras directly on the microscope tube. The Leitz firm still supply their Micro-Ibso attachment (new code, Mikas) as illustrated in Figure 20, which can accommodate not only the Leica camera but also their larger-size Makam camera attachment (Figure 21) which takes pictures 9 x 12 cm.

Bausch & Lomb, Zeiss,* and Reichert also supply small cameras

* After World War II, the original firm of Carl Zeiss, Jena, was divided into two separate firms, one located in East Germany and a new firm which was moved to West Germany and combined with R. Winkel. The microscopical apparatus of both firms is still marketed under the name Zeiss. In the United States the West German company goes under the name Carl Zeiss, Inc., 485 Fifth Avenue, N.Y.C., and the East German company is represented by the Ercona Corporation, 527 Fifth Avenue, N.Y.C. Old equipment products were apparently divided between the two companies. A piece of apparatus referred to Zeiss in the text may be the product of either organization.

for direct attachment to the microscope tube. These all follow the general pattern of the Leitz attachment, except for minor differences, and are designed to take both 35-mm. cameras and larger fixed-focus cameras. All are equipped with a split-beam viewing device by means of which focussing can be done and the image observed up to the time of taking the picture. Bausch & Lomb employ, instead of the viewing eyepiece, a ground-glass screen on which the image can be observed. Their large camera takes pictures $2\frac{1}{4}'' \times 3\frac{1}{4}''$. The Zeiss



FIG. 21. Leitz Micro Camera Attachment—Makam and Macca

size is 6×9 cm. and the Reichert 9×12 cm. The Bausch & Lomb Eyepiece Camera (Model N) is shown in both setups in Figures 22 and 23, and the Zeiss and Reichert designs in Figures 24, 25, and 26.

Micrographs as taken on motion-picture roll film by either of these cameras are shown, at full size, in Figure 27.

These small outfits have several advantages, but also certain disadvantages and limitations. Among the good features are:

(1) Economy of operation. The cost per exposure, when motion-picture film is used, is extremely low.

(2) The space required for the entire equipment is small. It can be stored almost anywhere when not in use, and quickly set up whenever wanted. Any place where a microscope can be used for visual purposes is satisfactory for photomicrographic work as well.

(3) The greatest value of the miniature camera to the science of photomicrography lies in its application to the photographing of living

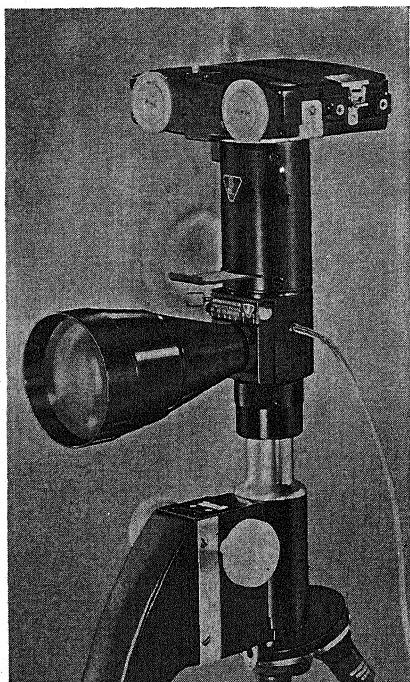


FIG. 22. Bausch & Lomb Eyepiece Camera, Model N, for 35-mm. Film

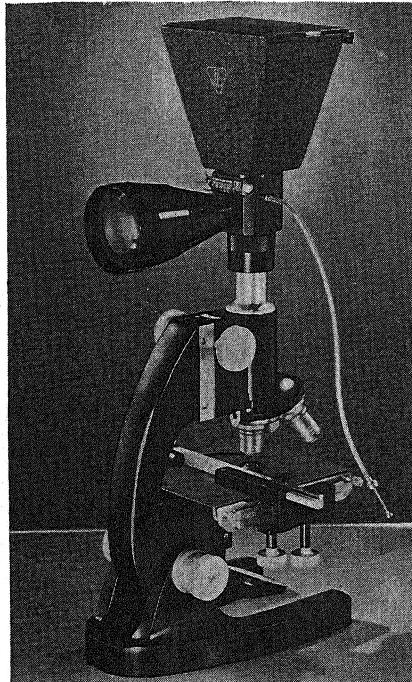


FIG. 23. Bausch & Lomb Eyepiece Camera, Model N, for $2\frac{1}{4}'' \times 3\frac{1}{4}''$ Film

microorganisms. The only alternative is the use of motion-picture equipment, which necessitates an elaborate and expensive setup and is not nearly so flexible in other ways. Motion pictures, even when taken on 35-mm. film, provide a picture limited to one frame size, as compared to the two-frame pictures taken with a minicam. Then they can be used as motion pictures only in large projection machines, whereas nearly everyone employs 16-mm. film for noncommercial

purposes today — a size entirely inadequate for general photomicrographic work.

(4) The roll-film camera is both convenient and rapid when a large

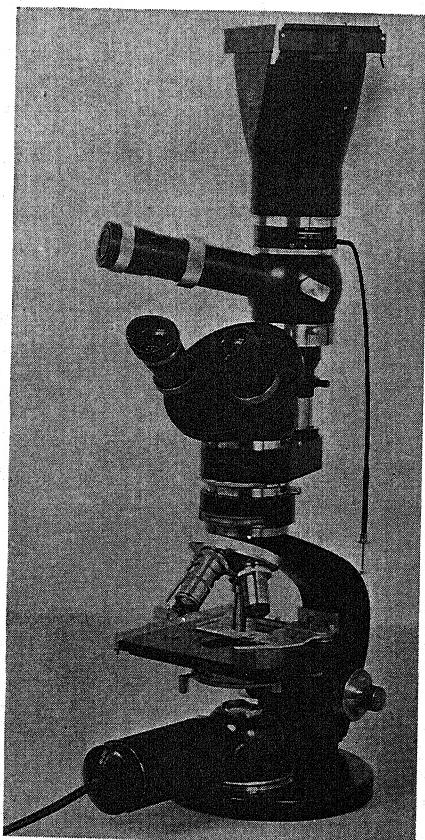


FIG. 24. Zeiss Micro Reflex Camera
Mounted on a Carl Zeiss Research
Microscope "W"

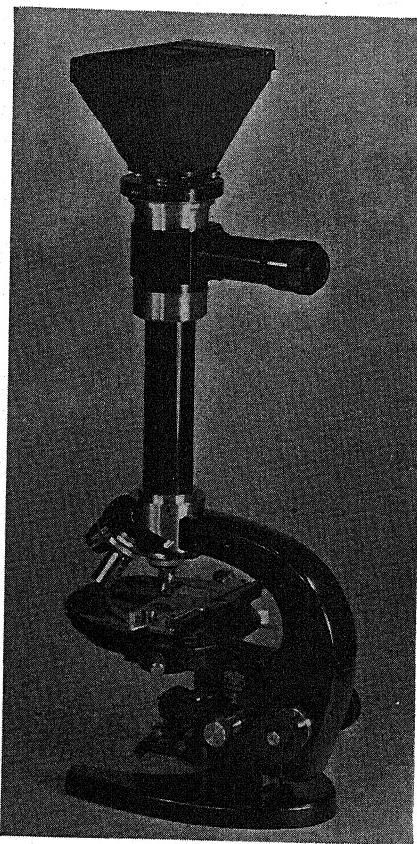


FIG. 25. Zeiss Jena (East Germany)
Attached Camera

series of photomicrographs in sequence, or under identical conditions, is required.

(5) If one continually uses a miniature camera for ordinary work, his proficiency in manipulative technique can be carried over to include photomicrographic work, when the same camera is employed for both purposes.

(6) Although the initial cost of the camera with its attachment is high, as compared with other simple photomicrographic outfits, if purchased *solely* for photomicrographic work embracing special problems, the camera can also be used for ordinary candid camera work. This may open up an entirely new hobby to its owner.

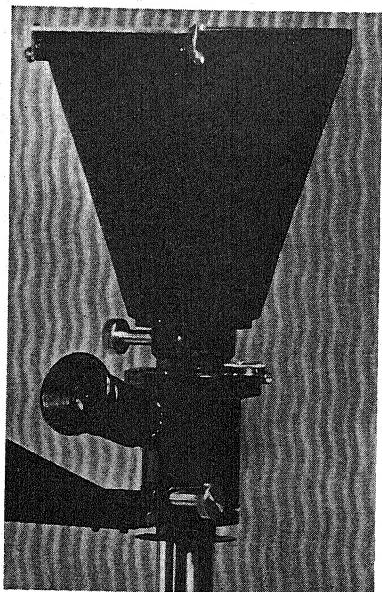


FIG. 26. Reichert Kam-V

subjected in projection, and the development of special projectors for miniature lantern slides, have been largely responsible and have helped to establish the miniature camera firmly in the photomicrographic field.

Contra these advantages, the following considerations should be borne in mind in deciding what type of equipment may be best suited to solving one's individual problems.

(1) Because of the small size of negative produced, an enlarger is required for the making of the final prints. (If the minicam is already owned and in use, presumably the enlarger is also.) Should it be necessary to purchase the camera especially for photomicrographic work, the cost of the enlarger should be included in the total outlay required.

(2) Considering the limitations to which this type of equipment is subject, it represents an expensive form of photomicrographic apparatus, if purchased for this purpose alone. The same amount of money, judiciously spent, will provide a far more flexible outfit.

(7) For those wishing to take photomicrographs in color, the miniature camera offers by far the most inexpensive approach. Kodachrome is relatively cheap in motion-picture roll film, as compared with professional cut film in the larger sizes. The great interest manifested in recent years in color photography has extended to photomicrography as well, making this one of the most important fields for the miniature camera in combination with the microscope. The enormous enlargement to which Kodachrome can be

(3) It is not practical to take, develop, and study single pictures, as can be done when plates or cut films are used. It is *possible*, should occasion demand, to do this, by wasting some film and going to the bother of reloading the unused portion.

(4) Various exposures on a single roll cannot receive individual treatment as to type of developer (soft or contrasty) and time of development. It is this possibility of individual treatment of each subject which puts the finishing touches to ideal photomicrographs.

(5) The type of emulsion and film characteristics cannot be changed from one picture to the next. All pictures on a roll of film must conform to the limitations of the particular film employed regardless of whether or not it is the best for the purpose.

(6) A small picture size necessitates rapid exposures, thus narrowing the latitude within which one must work. Fast exposures are ideal under ordinary photographic conditions, but in photomicrography the variables — as to the nature of subject, nature of lighting, amount of magnification, aperture of system, filter factors, plate or film characteristics, etc. — are such that short exposures should be avoided.

(7) There is an extreme lack of flexibility in the amount of magnification obtainable. This limitation is present in all photomicrographic cameras which have fixed projection distances, regardless of the size of picture taken. It can be compensated for, to some degree, by variation in the amount of enlargement used for the final print, but this may result in excessive enlargement under some conditions. To bring a negative made on motion-picture film up to a five-inch circle requires a five times enlargement. With a given combination of objective and eyepiece available, should an object be *just beyond* the size which can be included in the field of view with a fixed projection distance, the only recourse is to change to a lower magnification that will allow the entire object to be shown. Yet this combination may be such that the image of the object will only about half fill the negative. Thus to bring it to a full five-inch size will require, not a five times enlargement, but nearer ten. At the same time the objective giving the smaller image will probably possess a correspondingly lower numer-

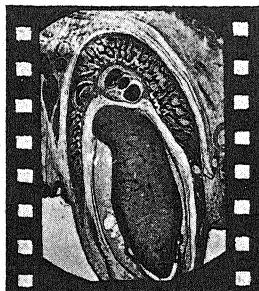


FIG. 27. A Photomicrograph Negative Made on Motion-Picture Film with a Miniature Camera

ical aperture not capable of full resolution when its eyepiece image is further enlarged ten times. Where a variable extension is present, it often suffices to reduce slightly the bellows length until the desired object is included. For instance, if one were using an extension giving a magnification of $50\times$, a reduction to $45\times$ or even to $48\times$ might accomplish the desired result; this would be achieved with the larger-apertured lens, yielding its correspondingly greater resolution.

(8) Except for the purposes of photographing fast-moving, living objects where an intense illumination results in their quick death, and the making of miniature Kodachrome micrographs, the use of roll-film miniature cameras should always be considered as strictly an amateur device. Such cameras cannot take the place of apparatus designed strictly for photomicrographic purposes, for serious research work.

(9) From the psychological point of view, there is one final objection to the use of roll-film miniature cameras in photomicrographic work. This lies in the very cheapness of an exposure and the ease with which exposures can be made. The result is that instead of trying to make each exposure as nearly perfect as it can be, one is tempted to take a dozen shots of each subject under slightly varying conditions, in the hope that one, at least, out of the lot, "ought to be good." This attitude is not conducive to the development of qualified photomicrographers. It is strongly recommended to all using minicams for this class of work that each picture be taken on the basis that *it must be good*, just as though each shot cost "a dollar per" instead of one cent.

The larger attached cameras are more practical from a photomicrographic standpoint in many respects. They are more flexible, and sheet film can be employed for color work; hence one is not limited to 35-mm. film when color pictures are wanted. Of course, such cameras are subject to the disadvantages of all types in which the projection distance is fixed; but with ample objectives and eyepieces of different focal length, they are still more flexible than the 35-mm. minicams. They are also of advantage for low-power macrographs, for which the miniature cameras are unsuited.

At the same time it must be admitted that in every instance where a separately mounted camera can be used, the objection to using the microscope as a support is eliminated and vibration reduced.

In the case of both small attached cameras and the more elaborate models, there is a considerable difference in the range of models offered by various manufacturers in each class. Some prefer to

standardize on a single model or at most two. Others, sensing a varied preference on the part of prospective purchasers for certain features, strive to meet requirements with a much larger selection. This holds true in microscopes as well, and extends throughout the gamut of photomicrographic equipment. Because of this it will be evident that many fine models available from some manufacturers must be omitted in discussing equipment and only such as are more likely to appeal to the largest number of users or display unusual features can be covered in illustrating what is available. Intending

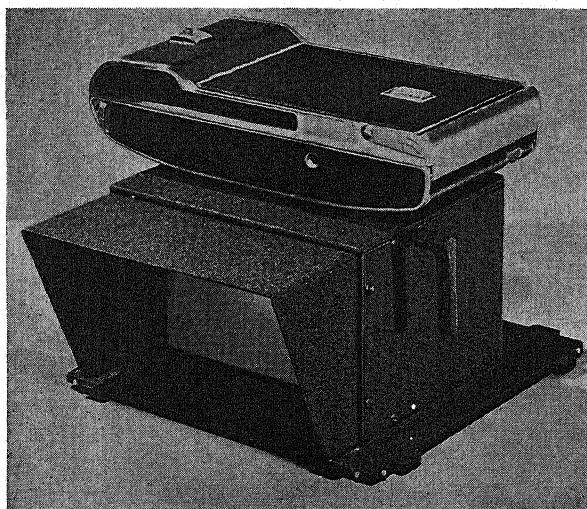


FIG. 28. Bausch & Lomb Polaroid Land Camera Attachment, with Reflex Viewer

purchasers should in all cases consult the manufacturer's catalogues before deciding what best suits their requirements, both technically and financially.

One development in the camera field which finds favor with many manufacturers of photomicrographic equipment is the Polaroid Land Camera as an accessory to their line of regular cameras. For those who desire a single picture for reference, or one of a transient condition under the microscope, this camera is ideal, since the picture can be exposed and a finished print be ready within one minute. It also makes possible the taking of a series of pictures to show interval changes that may be occurring.

Each manufacturer mounts the camera to suit the specific arrange-

ment of his camera backs. Bausch & Lomb's mounting, in combination with a reflex viewer, is shown in Figure 28.

Group 2—Universal Vertical Cameras

The simple vertical cameras of some years ago have practically gone out of existence. All developments, especially after World War II, were along the line of making even the simplest designs as nearly universal as possible. Mechanically they all follow a long-established general design—a baseboard arranged to mount the microscope and the lamp (in some cases an integral part of the unit equipment), a vertical rod or supporting column mounted on the baseboard adjustably supporting the camera, and the camera itself.

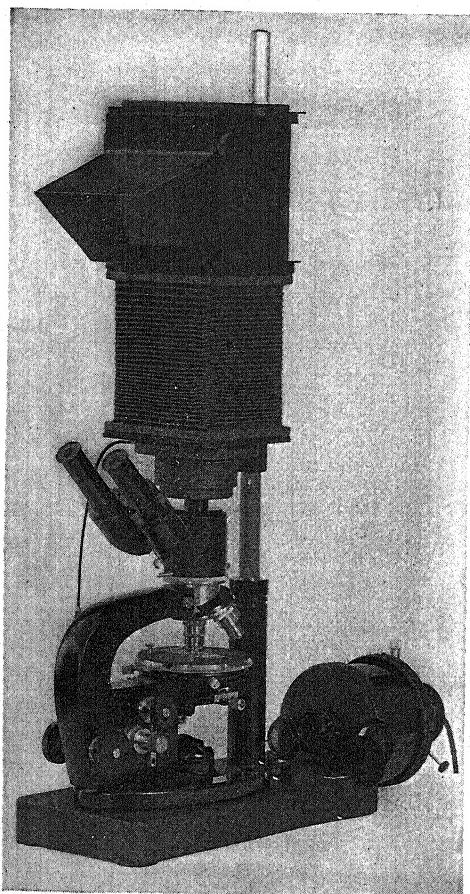


FIG. 29. Zeiss Vertical Standard Photomicrographic Camera

study the good points and limitations of each and select the one that most nearly fits his particular needs.

On comparing the designs of various manufacturers it becomes apparent that the term "universal" embraces widely different concepts of the requirements of photomicrographers. Actually no outfit is truly universal; every manufacturer's model has some features not incorporated in others, and each has certain limitations. Therefore it behooves the purchaser of an outfit to

A brief analysis of the general features incorporated in each design may be of value in directing attention to that most nearly meeting an individual requirement. This, followed by careful perusal of the manufacturer's catalogues, will make a suitable selection possible. Note that sometimes the manufacturer, in his desire to provide universal service, will include as part of the outfit items of equipment not required in a given purchaser's work and therefore adding an unnecessary expense. In other cases such included equipment may be the most important part of the outfit.

The Zeiss-Winkel Universal Camera. Basically this might be considered the simplest design available. The camera is of the ordinary bellows type, taking 9 x 12-cm. films, and the rod support on which it is mounted is 90 cm. in length, thus allowing for a wide range in bellows magnification. The rod swivels in the base so as to move the camera out of the way when using the microscope without it. This feature is common to the designs of all makers, although in some it is accomplished in different ways. The outfit is supplied with a high-efficiency lamp which is removable for macrostage work. A range of low-power photographic lenses is provided for mounting on the camera lens board when using

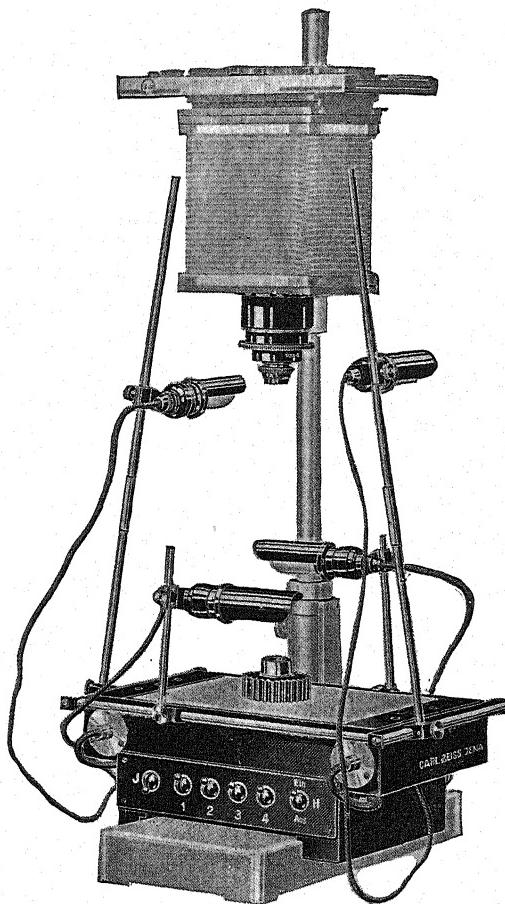


FIG. 30. Zeiss Macro Stage

the macrostage without the microscope. Figure 29 shows the setup for photomicrography, and Figure 30 the macrostage to which the

reflex back can be attached when required. Thus it will be evident that universality in this outfit consists of a complete range of magnification from a 1 : 1 ratio to the highest attainable with the highest-power objective and eyepiece available combined with a maximum bellows length. Other accessories, such as multiplicator back, Luminar low-power lenses, exposure meter, etc., can be supplied as desired.

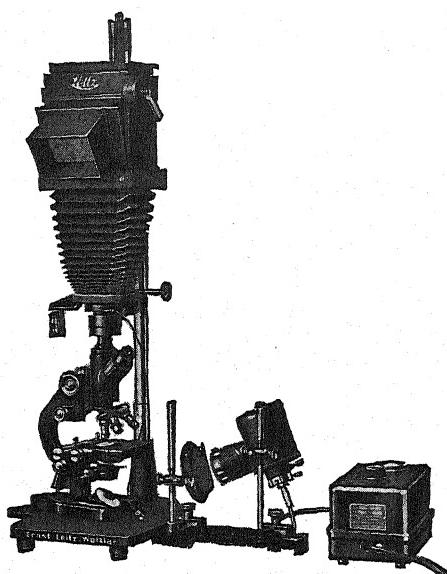


FIG. 31. Leitz Vertical Photomicrographic Camera, with Microscope and Lamp, in Position

split-beam telescope eyepiece. The large camera can be replaced by a minicam for 35-cm. film. For transparent macrophotographs a special macrostage is available, as well as a smaller camera for use with 35-cm. cameras which is of the bellows type to provide adjustable magnification. The cameras are arranged to take low-power lenses for use without the microscope. A special incident light illuminator with concealed lamps (see Figure 61), which attaches in front of the camera for photographing large opaque objects, can be supplied as required. The MA-IVb camera, equipped with the reflex attachment, is shown in Figure 31.

American Optical Company Camera. The basic design of the American Optical Company's photomicrographic camera, Model AO-682, is shown in Figure 32. The most obvious difference between it and the cameras previously discussed is the omission of the bellows camera and the substitution of a fixed-length camera. The universal

feature stressed in this design is its versatility in accommodating other types of cameras — 35-mm. minicams, the Bantam roll-film camera, and various types of camera backs. Among these are a back to take Graflex double plate holders, film-pack adapters, roll-film attachments and the Polaroid Land Camera back.

A focussing telescope eyepiece and universal shutter are integral

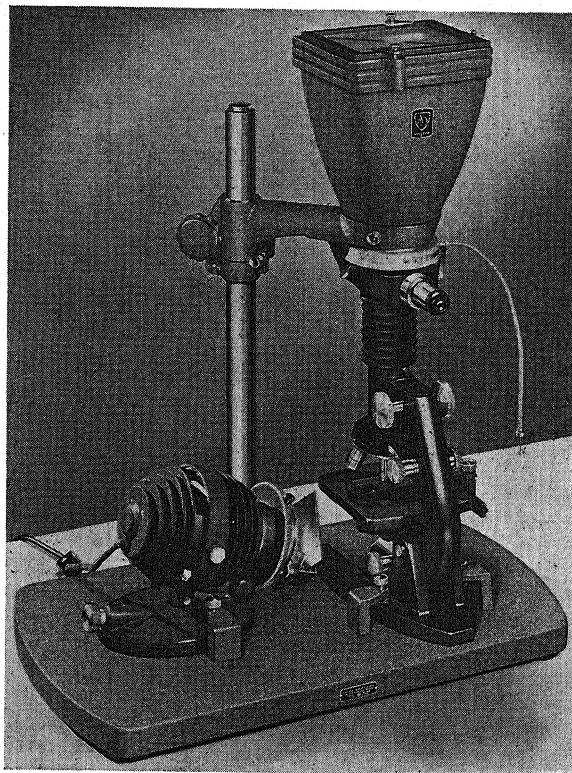


FIG. 32. American Optical Company Photomicrographic Camera No. 682, with the No. 735C Illuminator

parts of the outfit. Several advantageous designs are incorporated, among them a flexible bellows-type light trap for connecting the microscope to the camera, and rotation of the camera about the optic axis so that the object being photographed can be positioned on the film without the necessity of a rotating stage on the microscope. The baseplate is designed to accommodate the American Optical Company's high-efficiency #735C illuminator (Figure 32).

The greatest lack of universality in this outfit is the absence of an adjustable bellows length, regardless of which camera attachment is being used. Again, it has not been designed for low-power macrog-

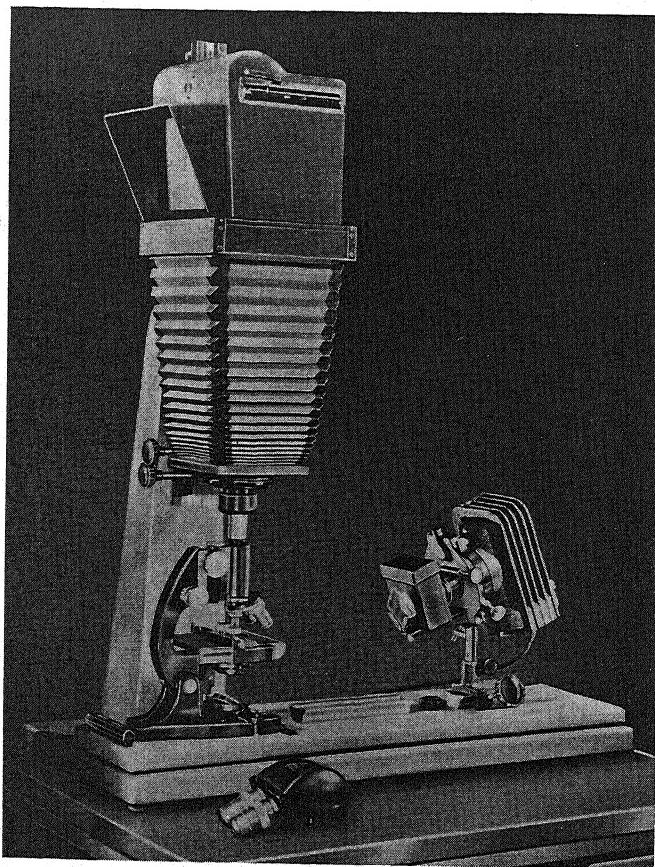


FIG. 33. Bausch & Lomb Large Vertical Camera, Model L, with the Ribbon Filament Lamp, Model PR-27

raphy, possibly because the American Optical Company do not supply a set of low-power photographic lenses for this purpose.

Bausch & Lomb Photomicrographic Equipment, Model L. Since Bausch & Lomb provide a fixed-focus eyepiece camera (Model N) equipped with a telescope eyepiece adapted for 35-mm. films and larger ($2\frac{1}{4}'' \times 3\frac{1}{4}''$) no attempt has been made to make their large

vertical camera (Model L) universal in this respect. Other features, however, provide for a greater field of usefulness in some lines than can be secured with the designs of other manufacturers. Their large bellows-type camera, taking 5" x 7" pictures, mounts on an adjustable vertical support, and the large base plate is provided with an auxiliary sliding top equipped with mounting means for the microscope and additional mounting strips functioning as an optical bench.

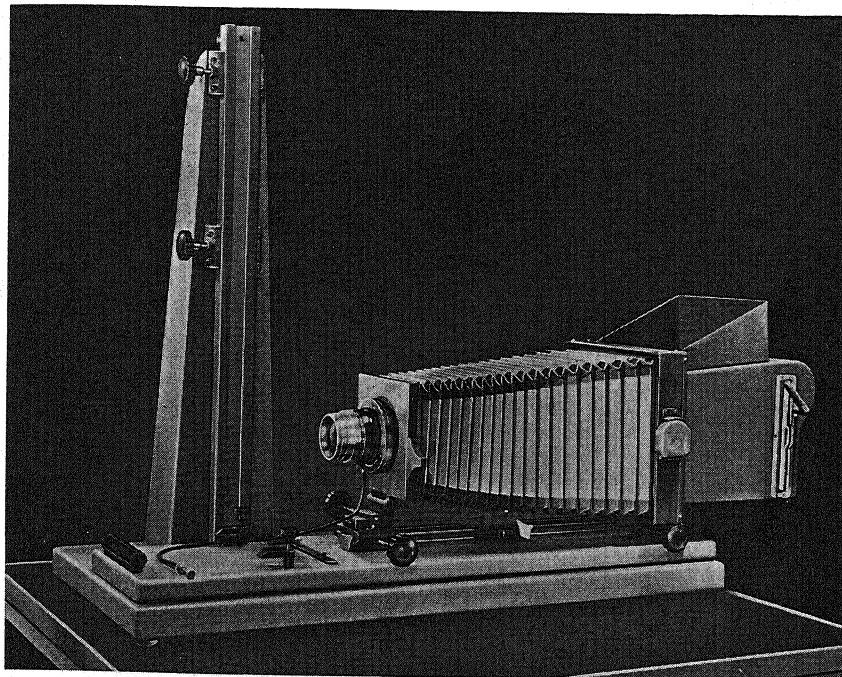


FIG. 34. Bausch & Lomb Photomicrographic Equipment, Model L—a Copy Camera with Reflex Back

This optical bench accommodates a wide range of accessory apparatus, including any one of several types of lamps, auxiliary stages, etc., enabling practically all types of photomicrographic work to be done. The sliding base makes it possible to move the microscope from under the camera for visual work. Even the camera itself can be removed from the vertical support and mounted on the horizontal optical bench, thus becoming a copying camera. A reflex back is available for work where it is an advantage. Included in the complete

equipment is a supporting cabinet and stool. Since each piece of equipment is separately priced, one can obtain such as are required for immediate use and add to them later as desired. This applies to such items as different types of lighting equipment, supports for

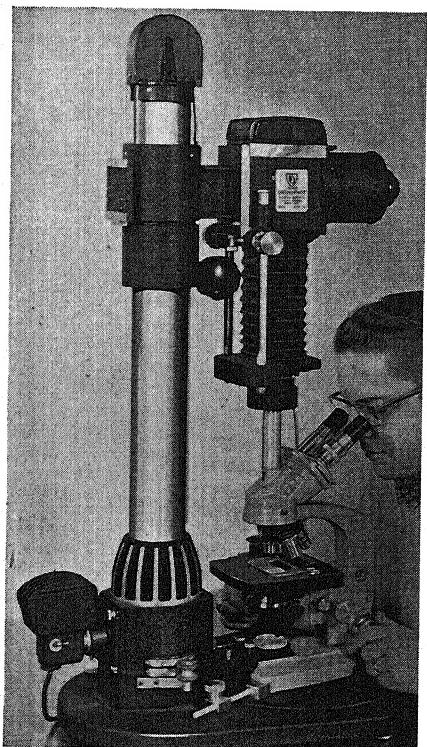
mounting large objects for low-power opaque and transparent photography, low-power photographic lenses, etc. The basic Model L camera is illustrated in Figure 33, and in the horizontal position for copying in Figure 34.

*The Orthophot.** The Orthophot represents a notable attempt to attain a truly universal vertical photomicrographic outfit. It has overcome some of the failures of other types of equipment to achieve this distinction. The basic equipment for photomicrography is shown in Figure 35. It includes, as a fundamental part of the outfit, the light source, an adaptation of the Ortho-illuminator shown in Figure 57 and described on page 81. The camera is of the bellows type, with reflex mirror, built-in focussing device, and interchangeable arrangements for various types of film holders, cut film, film pack, double plate

FIG. 35. Orthophot No. 1300, with American Optical Company Microscope Microstar

holders, and 35-mm. film holder. The standard camera takes $2\frac{1}{4}'' \times 2\frac{1}{4}''$ pictures, but camera backs to accommodate $2\frac{1}{4}'' \times 3\frac{1}{4}''$ are available. The entire apparatus is designed in sections, and by means of additional equipment can be used for low- and high-power photomicrography, gross objects, photocopying, microfilming, general pho-

* The Orthophot was designed and manufactured by Silge & Kuhne, 16th Street and Carolina Street, San Francisco, Calif., but its manufacture and distribution has been taken over by the American Optical Company as an item in their line.



tography, enlarging, cinephotomicrography, etc. Needless to say, to do all this requires considerable equipment beyond that considered the basic outfit.

The manufacturers were quick to realize the one lack in universality — the limited size of the picture that could be taken; accordingly they also furnish an alternative model, the Orthophot Special, which takes up to 4" x 5" pictures. This is the nearest to meeting every need that this outfit can attain.*

From this wide range of vertical outfits it is possible to meet the requirements of almost every type of work which one is ordinarily called upon to perform. Some of the outfits call for mechanical ingenuity to figure out how best to obtain the desired result in assembling the parts necessary to accomplish it.

To one who does not take pictures smaller than 5" x 7", who is accustomed to a bellows length of at least four feet, who prefers a horizontal camera where its use is possible, and who normally employs a high-intensity 500-watt light source but frequently replaces it with a 10-ampere arc, a mercury vapor quartz lamp, an electric sodium lamp, or an infrared light source, universality is still not achieved by any photomicrographic outfits standard at the present time. But it must be admitted such a microscopist is an exception to the rule.

Group 3 — Horizontal-Vertical Cameras

The only firm supplying at the present time a model coming under this classification is Bausch & Lomb, with their Type R outfit. This is a relatively simple model, consisting of a bellows-type camera mounted on a vertical rod hinged at the bottom so as to be swung into a horizontal position. The base plate is small, and no attempt is made to add refinements. The camera takes a 5" x 7" plate (or any smaller size with the help of reducing kits) and in its horizontal position can be used for copying, enlarging, etc. This design has been

* I have been advised by the manufacturers that although they list the Orthophot Special in their catalogue, the demand for it has not been sufficient to justify its continued manufacture and they are accordingly dropping it from their line. This is but one of many indications that the small cameras, especially the 35-mm. color-film cameras, are becoming increasingly popular in place of the larger models more adapted to real research and professional photomicrography. Inasmuch as the Orthophot line has been taken over by the American Optical Company, it is possible the 4" x 5" camera Orthophot Special may again be made available for those desiring a larger picture size.

standard with Bausch & Lomb for many years. It is shown in Figure 36.

Horizontal-vertical type cameras of more elaborate design have long been popular with advanced workers in England and Continental Europe. British equipment, both microscopes and photomicrographic outfits, although of high quality, has never invaded the American market to any great extent; but the two German firms of Leitz and Zeiss are well represented in all types of microscopical and optical equipment. Both have furnished horizontal-vertical outfits

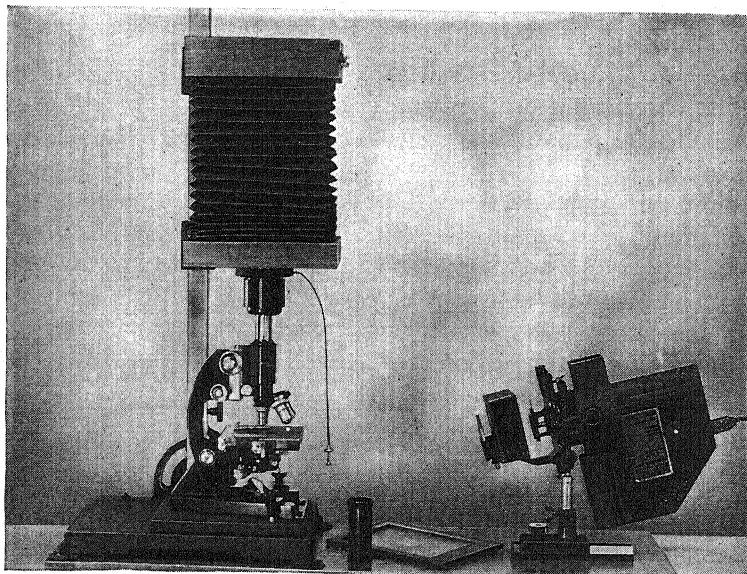


FIG. 36. Bausch & Lomb Horizontal-Vertical Camera (Type R) in the Vertical Position

adapted for universal service, and such are in common use at present in many laboratories. The only American firm which put out a competing research model was Bausch & Lomb.*

The present tendency toward vertical cameras (combined with restrictions brought about by World War II) has forced research models of the horizontal-vertical type off the market. The only exception which still defies the elimination of horizontal cameras lies in the metallographical field, where research outfits for this purpose still hold sway.

* Model GBVP, now discontinued.

However, not only because of the historical place horizontal-vertical equipment holds in the development of universal photomicrographic outfits, but because so many of these older models are still in use and regarded by their users as the only true universal cameras, they deserve a place in any work on photomicrography. It will suffice to point out their valuable features from the standpoint of the research worker desirous of the utmost in versatility.

The Bausch & Lomb equipment known as Model GBVP, of which there are large numbers still in use, is shown in its horizontal position

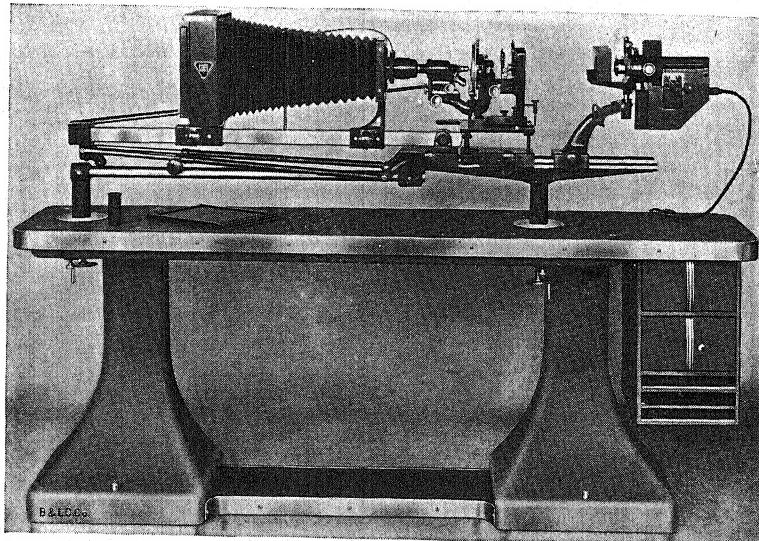


FIG. 37. Bausch & Lomb Photomicrographic Outfit GBVP, in the Horizontal Position

in Figure 37. The large 8" x 10" camera, with its long bellows (40-inch extension) and the extreme rigidity of the entire apparatus are plainly seen. When it is necessary to employ the camera and microscope in the vertical position, the rod with its three-point support is elevated as illustrated in Figure 38. The only limitation noticeable in this design is the relatively short space allowable in the optical bench between the light source and the microscope.

Leitz furnished a somewhat similar model, also mounted on a heavy unit base of very sturdy construction, well designed as a universal outfit; but relatively few of these equipments reached the American market.

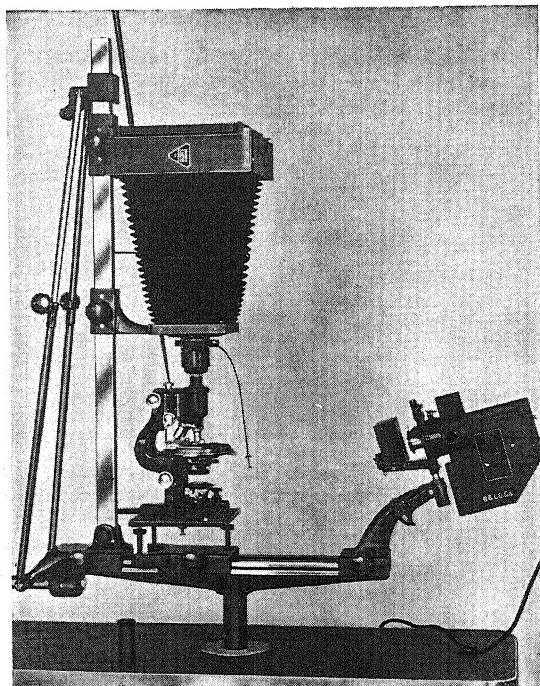


FIG. 38. Bausch & Lomb GBVP Camera in Vertical Position

The Zeiss 18 x 24-cm. horizontal-vertical outfit is also similar in general design. It is illustrated in its two positions in Figures 39 and 40. Of interest in this design is the long 1-meter optical bench, which provides great flexibility in operation and permits the use of other specialized equipment. The heavy cast-iron base on which the

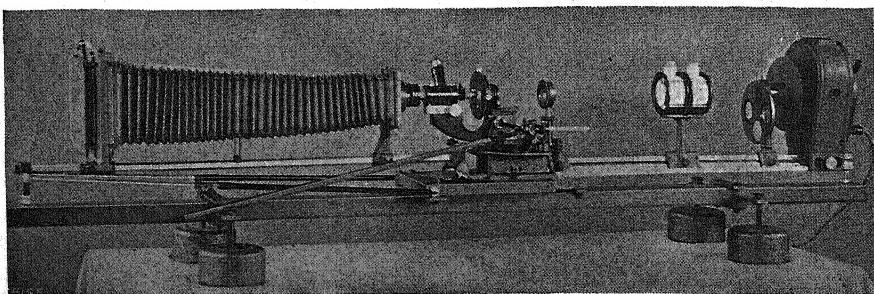


FIG. 39. Zeiss Horizontal-Vertical Camera in Horizontal Position

interchangeable sole plates for holding the microscope are mounted carries one end of the optical bench and also the camera supporting rod with its rigid three-point support. When used in the vertical position the camera can be swung back at an angle from the vertical support, against an adjustable stop, to make the microscope usable for visual work. The camera rod is graduated to a length of 112 cm. This Zeiss model represents the greatest possible degree of universality. For metallographic work, a second 1-meter optical bench

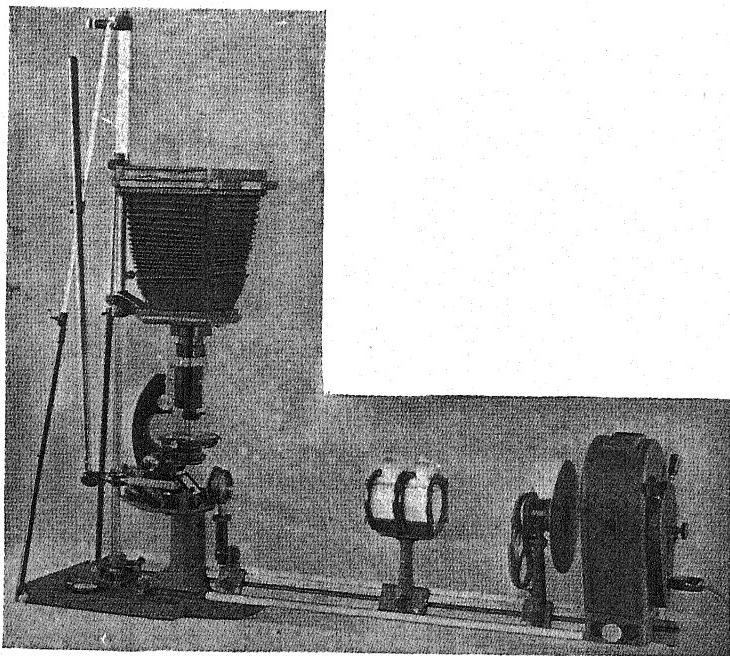


FIG. 40. Zeiss Horizontal-Vertical Camera in Vertical Position

can be mounted to the supporting base (with a suitable support at the outer end) at right angles to the outfit, as shown diagrammatically in Figure 138, page 226, to carry any of the auxiliary equipment and light sources usable on the main bench.

The camera can be used for copying, enlarging, and reducing and for macrophotography without a microscope. This is the only camera capable of utilizing the large 7-inch collimating and collecting condensers as furnished by Zeiss. These are ideal for photographing such objects as entire brain sections, full size or larger. Any type of

camera back can be adapted for mounting in place of the standard plate holders. Ciné cameras and 35-mm. film cameras can be mounted by means of special supports on the camera rod, the camera being moved to the outer end for this purpose. The only limit to complete universality of this outfit is space requirement. If equipped for metallographic work it requires an area of about 9' long by 5' wide, plus ample space to walk around it.

There will be a question in the minds of many microscopists as to the need for horizontal-type cameras, in view of the great range in design of so-called universal vertical cameras and unit-type outfits. Assuming that space is not a limiting factor and that one is called upon to do photomicrographic work in every field where the microscope is employed, the advantages of a horizontal outfit are numerous. Among these can be mentioned:

1. The simplicity of design, as compared with the numerous pieces of equipment necessary to accommodate the average vertical camera for all types of work. This means an appreciable saving in initial expense.
2. The advantage of having the optical axis a straight line from the light source to the center of the camera ground glass and elimination of the mirror, thus simplifying the alignment.
3. A long optical bench accommodating all sorts of auxiliary equipment — an obvious feature of a horizontal outfit.
4. The possibility of using a long camera — a decided advantage. A larger camera size is also an obvious possibility.
5. The fact that no reflex back is required. The image can be viewed directly on the ground-glass screen.
6. The fact that when several people need to view the image simultaneously, the ground-glass screen can be removed and the image projected to a distance on a large screen.
7. Flexibility as to light source. A horizontal outfit is especially adapted for interchangeability of all types of light sources — tungsten bulb, arc light, electric sodium lamp, mercury vapor quartz lamp, infrared source, high-frequency spark for fluorescence, etc. With suitable rider supports for the optical bench, an instantaneous change can be effected by merely lifting off one lamp and substituting another form of illumination.
8. Availability for general work. Having the camera horizontal makes it available for copying charts, etc., either reducing or enlarging as required, making lantern slides, photographing opaque

objects, and all such types of work. For these, an easel mounted on a rider and a light box with indirect illumination are inexpensive adjuncts.

9. Interchangeability. The microscope can be replaced by regular photographic lenses mounted on the camera lens board, while the long camera length provides great flexibility.

If the camera is of a fixed horizontal type, it is subject to but one limitation, i.e., the occasional need for photographing objects in fluid or slides which must remain in a horizontal position because of object movement. It is because of this restriction that horizontal cameras must be capable of operating in a vertical position as well. Adapting a camera to function in both positions involves but little additional expense. It is to be regretted that some manufacturer does not realize the numerous advantages of the combined horizontal-vertical outfit and make one available for those who would find it best adapted for their work.

Group 4 - Self-Contained Universal Outfits

Recent years have witnessed a radical departure in the design of photomicrographic apparatus. Unit outfits are rapidly superseding many of the older, more conventional cameras. The Leitz Company were the pioneers in this field when they brought out their Panphot Universal Microscope. They were quickly followed by Zeiss, with their Ultraphot, and in England by the Vickers Projection Microscope. So far American firms have not followed this trend toward a compact universal outfit.

The original purpose in designing this equipment appears to have been to provide a microscope and photomicrographic equipment, self-contained and permanently aligned, which could be used for every sort of work by technicians not possessing a basic knowledge of all the principles involved in the various types of work they might be called upon to perform.

The microscope can be used visually for transparent work with transmitted light; as a polarizing microscope; with vertical top illumination, for metals; with oblique top illumination; as a fluorescence microscope; for dark field, etc.; and drawing, projection, or photography can be performed under each condition. The change-overs in the lighting systems required for these various conditions of operation are all provided for in the design of the apparatus. They can be

made more or less automatically, without the possibility of misalignment or improper setup.

Space requirements for these universal models are small, a 2' x 3' table area being ample for any of them.* It must not be assumed, however, that size reduction has brought about a corresponding lowering of the cost, for these universal outfits are anything but inexpensive, especially if purchased with all the attachments necessary for every type of work.

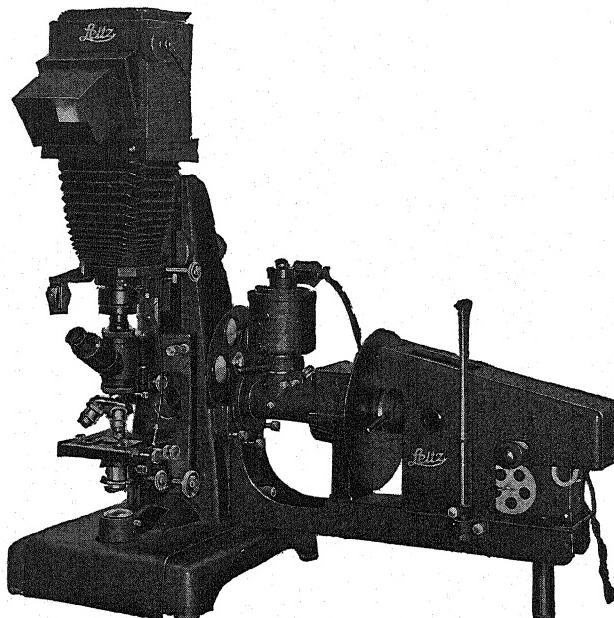


FIG. 41. Leitz Panphot

The basic model of the Leitz Panphot for general work is illustrated in Figure 41. It would not be feasible to attempt to show the appearance of this, or other makes of universal outfits, in all the various set-ups of which they are capable. For some work the changes are minor, but for others, one would hardly recognize the combinations as having any relation to each other. For work with transmitted light, the arrangement must be such that the illumination is from below the stage.

* Contrast this with the author's large horizontal-vertical outfit, with its duplexed 1-meter optical benches, requiring a floor space about 5' x 9'.

For top illumination, metallography, etc., light enters the various illuminators from the rear, above the stage. The standard plate size of the Panphot is $3\frac{1}{4}'' \times 4\frac{1}{4}''$.

The original design of the Panphot, with but minor changes, is still standard with Leitz. However, since World War II there appears to have developed a conviction on the part of manufacturers of self-contained universal outfits that intermediate, less elaborate models are required to bridge the gap between the research microscope for strictly visual work (or used with some type of photomicroscopic camera) and the elaborate self-contained models. Accordingly Leitz, Zeiss, and Reichert, all of whom make the elaborate outfits, provide an intermediate, less complicated (and less expensive) design. These are, in effect, glorified models of standard microscopes, with built-in illumination and means for attaching photomicrographic equipment, as well as attachments for doing practically all types of microscopic work. The Leitz model, known as the Ortholux is shown in Figure 43.

The corresponding intermediate model of Zeiss (Model W) is shown in Figure 24, page 45. When assembled as a standard microscope it does not differ materially in appearance from a conventional microscope. Its chief characteristic lies in the complete interchangeability of every part. Thus one can build up the

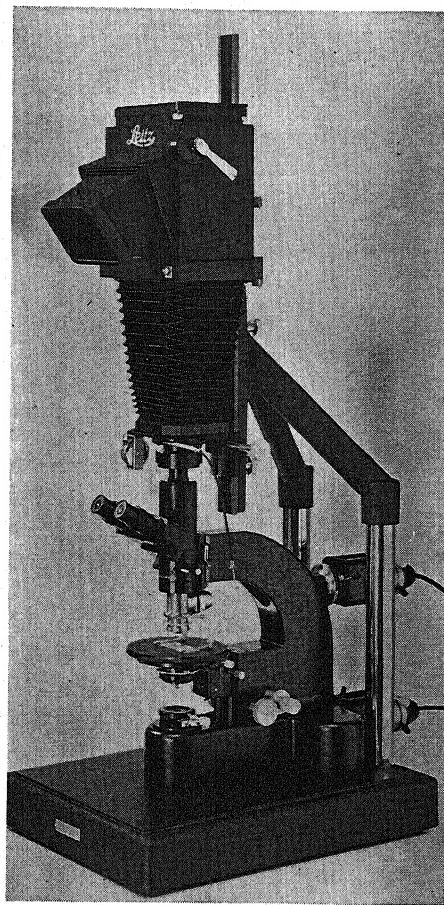


FIG. 42. Leitz Ortholux Microscope Mounted on Aristophot II Photographic Equipment

particular instrument adapted to his specific requirements. Several types of stages are available, as well as other components, hence the stand can be assembled for petrographic, metallographic, and photomicrographic work as well as for phase, light and dark field, etc.

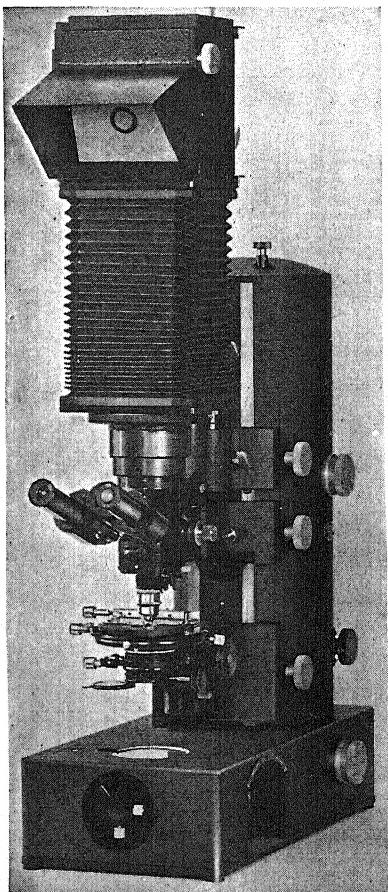


FIG. 43. Zeiss Ultraphot

est quality of workmanship, both mechanically and optically.

Undoubtedly the most radical departure in microscopic and photomicrographic equipment is the Vickers Projection Microscope* shown in Figure 47. It is an inverted form of microscope, with the stage on top and the objectives pointing upward, as in the Le Chatelier

* Manufactured by Cooke, Troughton & Simms, York, England.

The original Zeiss universal outfit, known as the Ultraphot, is shown in Figure 43. It has been replaced by the Ultraphot II, seen in Figure 44. This represents a radical departure in design from the original Ultraphot and incorporates several new features.

A third European company (long known for their fine microscopes) to invade the universal microscope field since World War II is Reichert, of Vienna. They also have an intermediate model, called the Zetapan, shown in Figure 45. Their self-contained universal outfit (Code MeF) is illustrated in Figure 46.

It is impossible to do justice to the various features incorporated in the instruments of the various manufacturers. Naturally each possesses features of merit, and as could be expected, they are all high in price. Those interested in instruments of this type should study the catalogues of all, comparing various designs with price.

All can be expected to be of high-

metallurgical microscope. The reason for this appears to be two-fold — first, it is primarily intended for a metallographic outfit, and second, it employs a reflecting system to extend the projection distance and enable one to view the image at a comfortable angle, looking downward. Like the Leitz and Zeiss models, it requires marked changes in the setup for accomplishing various results. Alteration of the projection distance is effected by varying the position of the reflecting mirror, each inch of movement being equal to two inches in the projection distance. An ingenious but complicated arrangement is provided for automatically changing the mirror angle and maintaining the plate normal to the projection axis as the projection distance is varied. This is shown diagrammatically in Figure 48. The mechanical construction is very rugged; it is claimed that objects up to fifty pounds in weight can be supported by the stage. The outfit itself weighs considerably more than two hundred pounds. Special equipments for all types of work, including polarized light, etc., are available.

Whether future trends in photomicrographic equipment will continue along these lines, to the final elimination of the older research models, is still an open question. For those starting with a universal outfit, it may seem difficult to graduate to the older optical-bench apparatus; on the other hand, one who has grown up with the latter could probably never be content with even the most thoroughly equipped universal microscope. It is, in the last analysis, largely a matter of personal inclination, but fashion has some part in establishing habits and changing tastes, and so too does the commercial drive to develop new lines of instruments. Since the need for the kind of apparatus represented by older research models is a lasting one, we

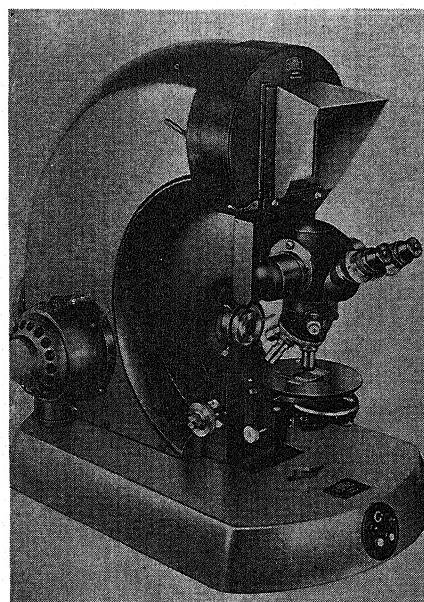


FIG. 44. Zeiss New Model Universal Microscope, Ultraphot II

may expect that such instruments will not altogether disappear from practice.

Group 5—Specialized Photomicrographic Apparatus

Certain types of photomicrographic work are so highly specialized as to require radically different setups, as contrasted with the more conventional lines of work. In some cases these setups may involve only extra equipment and slight modifications of standard outfits to

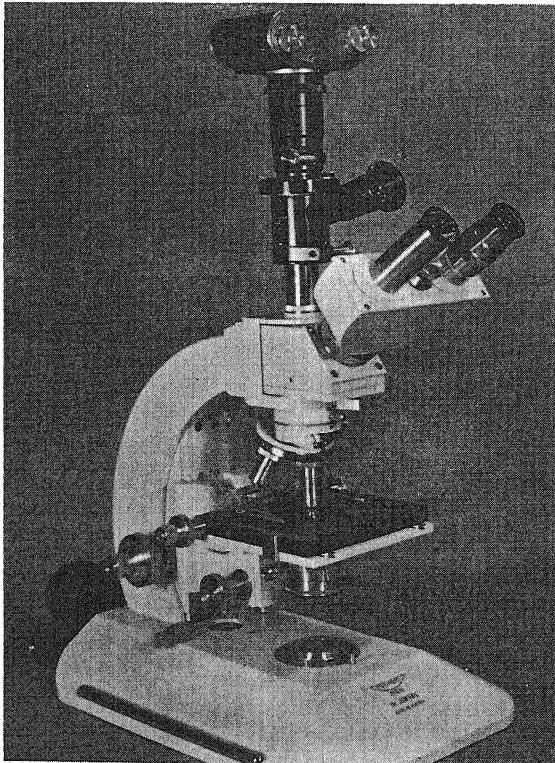


FIG. 45. Reichert Photomicrographic Camera, Zetapan

adapt the latter to the class of service required. In others, a complete outfit, designed specifically for one line of work, is necessary.

The recent trend on the part of manufacturers, especially those putting out the universal outfits, is to provide the necessary attachments and accessory apparatus arranged to mount on, or at least utilize, some portion of their basic equipment. For some classes of work this is quite satisfactory, but in others it is obviously only a makeshift.

Among the types of work which can be considered as falling within this general classification can be mentioned: metallurgical photomicrography; photography in the ultraviolet region; colloidal work with the slit ultramicroscope; spectrographic and fluorescence microscopy; motion-picture photomicrography; and stereoscopic photomicrography.

Special apparatus, as well as complete specialized equipments, to handle all these kinds of photographic work are made by various manufacturers. As each type of work will be discussed later, with the techniques adaptable to each, it will save duplication to cover both the apparatus itself and the problems arising in its use at the same time. Figures and descriptions illustrative of this specialized equipment will accordingly be found in Chapter 5.

In procuring outfits for specific purposes, where there exists a choice of either a unit equipment, designed to do a single type of work in the best possible manner, or adaptations of universal outfits through the employment of accessory equipment, each case must be considered on its own merits. No hard and fast rule can be made to cover every condition. In general, however, it can be stated that where only one particular line of work is contemplated, or where the extra expense of duplicate equipment or space limitations are not factors, more satisfaction will result from having unit apparatus for each highly specialized line of work.

Equipment for Low-Power Photomacrography

Photomacography enters a region of magnification not usually required, or employed, for visual work with the compound microscope.

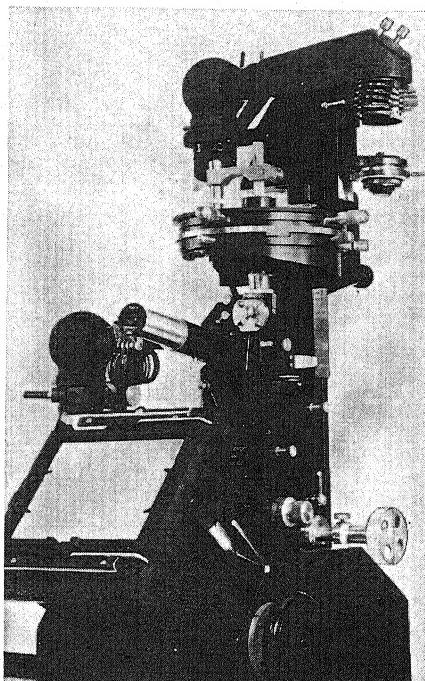


FIG. 46. Reichert Universal Model (Code MeF) Photomicrographic Outfit

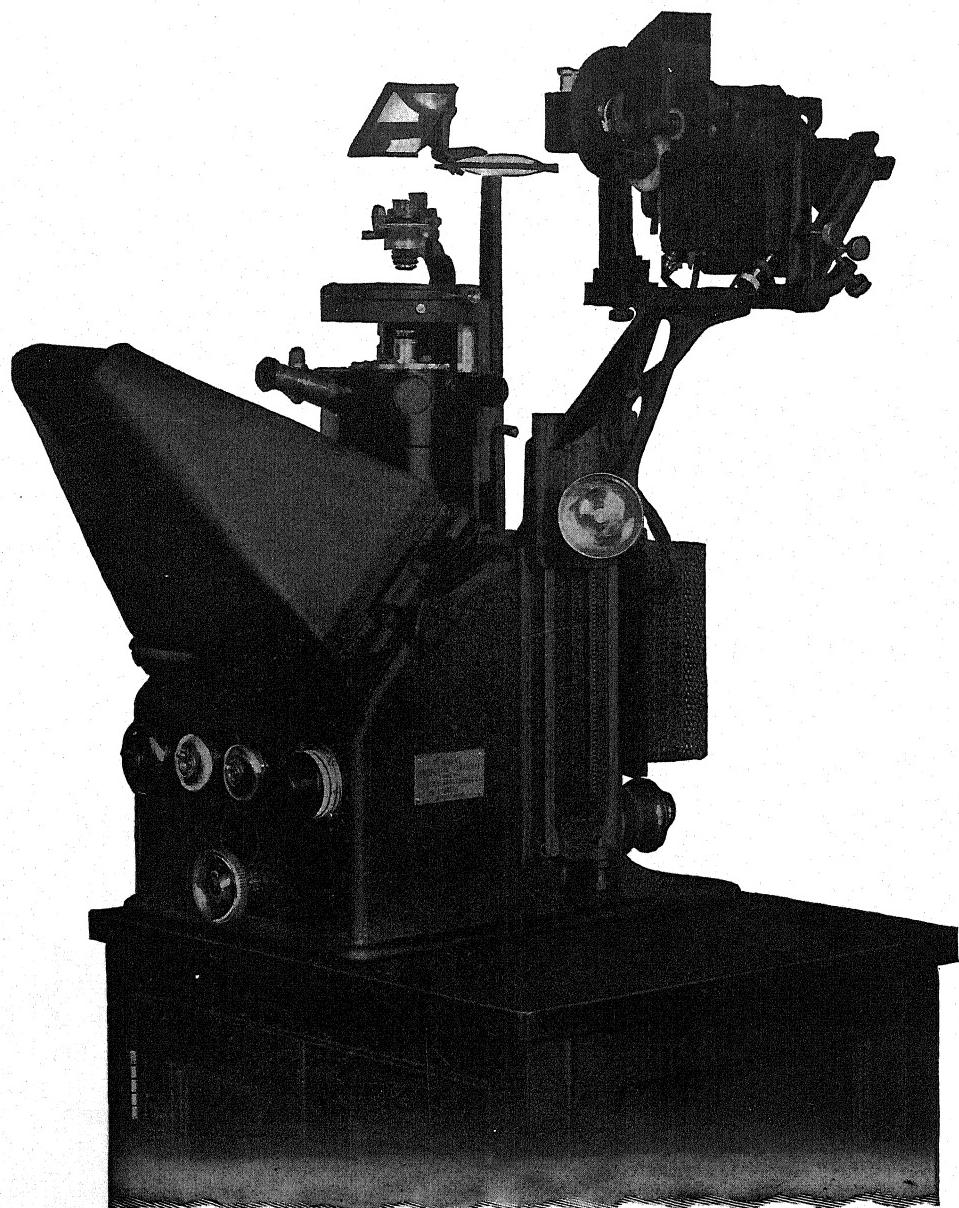


FIG. 47. Vickers Projection Microscope

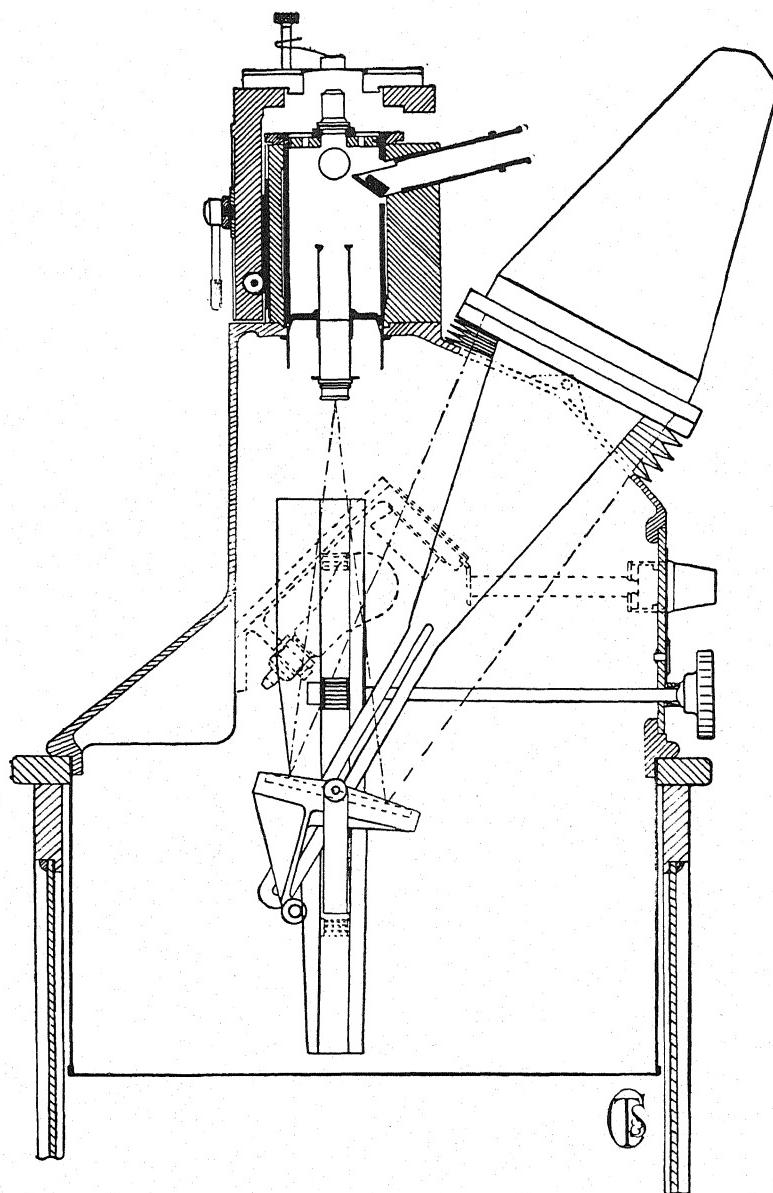


FIG. 48. Diagram of the Construction of the Vickers Projection Microscope

This includes the range from full-size pictures, up to possibly 20 diameters, where the average lowest-power combination of objective and eyepiece usually starts.

Thus a series of photomacrographic lenses, designed to work without eyepieces, must be available. These function as ordinary photographic lenses, the only difference being that they are computed so that their longer conjugate focus is at the back of the lens, the shorter focus being in front. The average lens of this type, regardless of focal length, usually works at a full aperture of $f:4.5$, or approximately a numerical aperture of .10. They are then provided with iris diaphragms, for reducing the aperture and increasing the depth of focus. The field of such lenses is quite flat over the entire working area, in this respect differing materially from the curved field so characteristic of compound microscopes when ordinary eyepieces are employed.

The entire range of magnification involved cannot be covered by any single lens; a battery of several is necessary, although with a complete series and a long bellows it is possible to extend the magnification well beyond that provided by a low-power objective in combination with a low-power eyepiece.

Each manufacturer designates his lens series by a special trade name, and the specific focal lengths of the lenses constituting the series vary somewhat with different manufacturers, although not to any material extent.

The Bausch & Lomb series is designated by the name Micro-Tessar. The set includes four lenses, 72 mm. ($2\frac{7}{8}$ "), 48 mm. (2"), 32 mm. ($1\frac{1}{3}$ "), and 16 mm. ($\frac{2}{3}$ ") focus, respectively. Figure 49 shows the complete set, together with the corresponding condensers required for transmitted-light work, and the barrel mount employed when a microscope is not used.

For still lower magnification than that provided by the 72 mm. Micro-Tessar, the series can be supplemented by the regular II B Tessars, working at $f:6.3$. These are made in various focal lengths extending down to $4\frac{5}{8}$ ".

Prior to World War II the American Optical Company (under the name of the Spencer Lens Company) marketed a set of low-power photomacrographic lenses known as Micro-Teleplats. The four lenses in the set are similar in equivalent focus to the Bausch & Lomb set except that the lens of shortest focus is 24 mm. (1 inch) instead of

16 mm. ($\frac{2}{3}$ inch). The manufacture of this set has now been discontinued.

Leitz lenses of this type are called Summars and Micro-Summars. The set includes five lenses, 100 mm., 80 mm., 65 mm., 42 mm., and 35 mm. focus. In addition to these highly corrected lenses, Leitz also puts out a series not so well corrected, at a lower price. These are designated Milars. In equivalent focus they correspond to the Summars except in the case of the two shorter-focus lenses, which are

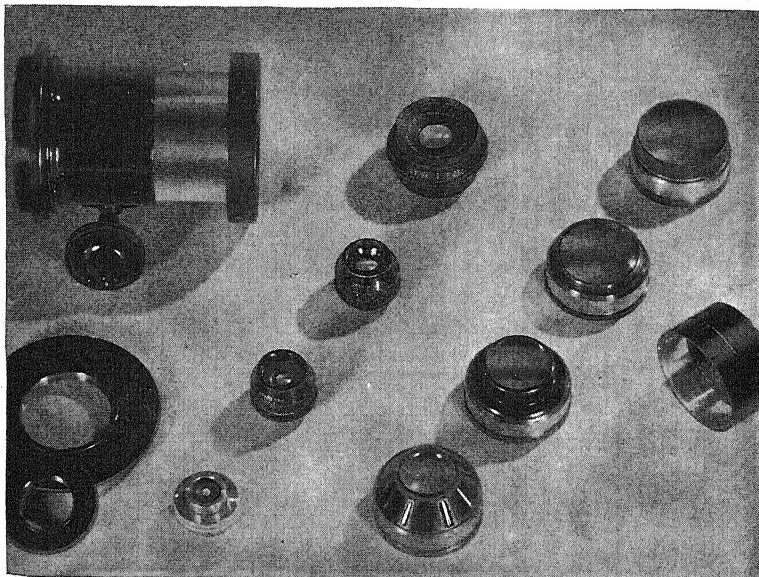


FIG. 49. Bausch & Lomb Micro-Tessars with Camera Lens Board Mount and Special Low-Power Condensers

40 mm. and 30 mm. in the Milar series, instead of 42 mm. and 35 mm., as in the Micro Summars.

For many years Planars were standard with the Zeiss Company. They were made in 100 mm. (4"), 75 mm. (3"), 50 mm. (2"), 35 mm. ($1\frac{1}{2}$ "), and 20 mm. ($\frac{4}{5}$ ") sizes, all working at $f:4.5$. This series of five lenses was replaced by a new series, known as Mikrotars. There are eight lenses in this series; instead of all working at a uniform aperture, the aperture is progressively increased in the shorter-focus lenses and reduced in the longest-focus lens. Increase in the f ratio as the focal length decreases is consistent with standard practice

on all other microscope objectives and enables much higher magnifications to be employed without introducing empty magnification. The optical data on the Mikrotars, as given by the Zeiss Co., is on page 76. Six of the lenses in the Mikrotar series are illustrated in Figure 50.

With all the manufacturers, it is standard practice to equip such lenses, where the size permits, with the Royal Society screw thread so that they may be mounted on the microscope just as though they were objectives. This is feasible up to about 60 mm. ($f:4.5$) lenses. Beyond this point the diameter of the lenses increases rapidly. Unless the mi-

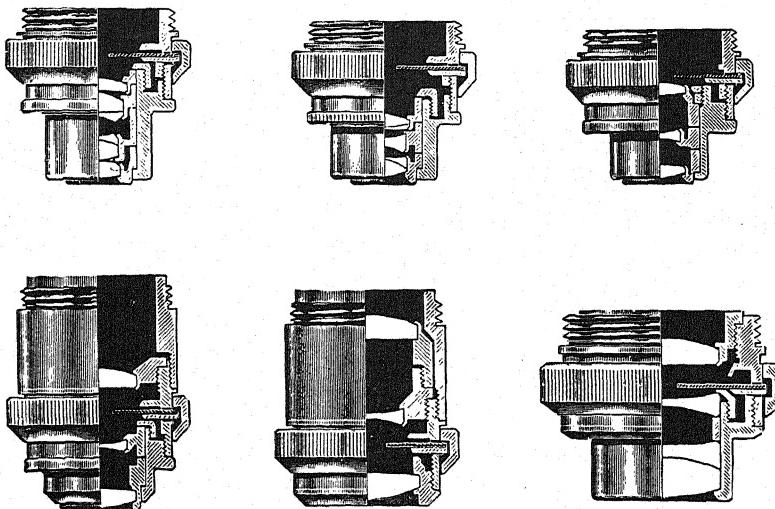


FIG. 50. Zeiss Series of Mikrotars, in partial section

croscope is of the large-tube type and provided with means for carrying the long-focus lenses, the latter cannot be used on the microscope at all, but must be mounted directly to the front board of the photomicrographic camera. The old Zeiss method of mounting the large lenses on the big-tube microscope is very practical, because it provides so much additional space between the stage and the lenses. It consists of a funnel (Figure 51) screwing into the top of the tube, and threaded at the bottom to accommodate the largest-diameter lens. As the funnel is materially shorter than the tube itself, the lenses are thus carried within the tube, entirely out of sight, providing an additional space of a couple of inches between the lens and stage, for any

given position of the tube. The nosepiece and bottom tube ring into which it mounts must, of course, be removed when this setup is employed. To do this quickly, a large tube sliding changer is available. In the later models of stands, where the entire tube is made interchangeable for monocular and binocular purposes, the funnel is available as a funnel tube accessory.

In all cases where low-power photomicrographic lenses are employed in transmitted-light work, on transparent objects, it is necessary to use a substage condenser suited to the particular lens in question, in order to provide critical lighting of the full field being covered. Each manufacturer accordingly provides such condensers as standard equipment. Those of Bausch & Lomb are included in Figure 49. These condensers are not required for incident illumination of opaque objects.

For many purposes, especially when the longer-focus lenses are used, a microscope is not necessary; it may even be a handicap. The omission of the microscope means that the photomicrographic lenses must be mounted directly on the lens board of the camera. To this end, most manufacturers provide some type of fixture, preferably with focussing means incorporated in it, for carrying the lenses on the lens board. Bausch & Lomb's device for this purpose is seen in Figure 49.

For vertical cameras where the lens is within easy reach of the hand, while one is examining the image on the ground glass, a simple screw focussing arrangement is ample. When, however, a long, horizontal camera is employed, some extension-rod device for distant control becomes necessary. Zeiss' lens-board focussing mount for use with

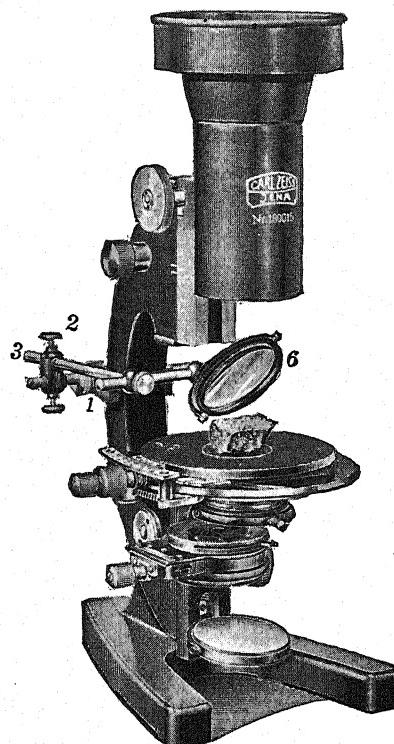


FIG. 51. The Zeiss funnel for use with the Mikrotars, shown in place on the microscope

OPTICAL DATA OF THE MIKROTARS

Focal Length, cm.	Aperture Ratio	Numerical Aperture	Theoretical Lower Limit of Useful Magnification	Scale of Reproduction for 1-Meter Distance of Image from Principal Point	Approx. Size of Field of View, mm.
1	$f:1.6$	0.31	155X	99:1	3.5
1.5	$f:2.3$	0.21	109X	66:1	5
2	$f:3.2$	0.15	78X	49:1	7
3	$f:4.5$	0.11	55X	$\begin{cases} 32:1 \\ 21:1 \end{cases}$	15
4.5					
6	$f:6.3$	0.08	40X	$\begin{cases} 16:1 \\ 10:1 \\ 7:1 \end{cases}$	20 30 60 80
9					
12					

the large horizontal outfits is shown in Figure 52. Rotation of the side spindle, by means of the distant control rod, moves the lens through the operation of a spiral thread on the lens mount.

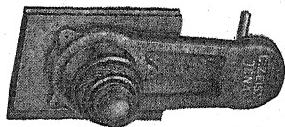


FIG. 52. Zeiss Focussing Mount on Lens Board. A long focussing rod is attached to the spindle shown at the right-hand side.

the same in both. The issue is solely the intensity of the light source required. For photography the illumination must be ample to enable accurate focussing to be done, even when color filters of very low transmission characteristics are employed, and also to keep exposure times within reasonable limits. A light that is ideal in intensity for visual work will be found to yield practically no image on the ground glass, even at a ten-inch projection distance, when a green screen with a filter factor around 10 is introduced in the light train. On the other hand, when a light source sufficient to give a good image on the ground glass is being used, the visual image will appear of dazzling intensity and the eye should not be placed at the eyepoint, as injury

Illumination Equipment

Illumination problems involved in photomicrography with transmitted light are quite different from those occurring with visual work alone. The principles of correct illumination are not at issue; they are

to the eye might result. This is why special sources of illumination are supplied for photographic purposes.

In the early days of photomicrography, three brilliant sources of light were considered suitable: the oxyhydrogen limelight for those without electricity, and the arc lamp and the Nernst lamp for those who were fortunate enough to have electric current.

The limelight is now a relic of the past; so also is the Nernst lamp. It is rather surprising that some enterprising manufacturer has not developed a photomicrographic lamp upon the Nernst principle, for it is a beautiful light source, comparable to many of the special high-intensity lamps on the market. Its only drawback is the preliminary heating required for the glowers, but this could be easily overcome by proper design.

The arc lamp, though always accepted as a suitable light source, has had its objectionable features, not the least of which is the inherent tendency of the arc to wander, shifting its position out of the optic axis. Proper design has overcome this objection, so that today the arc lamp is thought of first when maximum intensity of illumination must be provided.

The simplest form of the arc lamp is the hand-feed type, usually of about five amperes current capacity when used on direct current and ten amperes on alternating current. If the lamps are properly designed they can be made to work equally well on either d.c. or a.c. The higher operating amperage is necessary with the latter, since the effective intensity is correspondingly lower because the voltage passes through zero twice in each cycle.

As hand-feed lamps are not very satisfactory for photomicrographic work, all manufacturers supply, at a somewhat higher cost, mechanical-feed lamps. The lamps are mounted in suitable housings, properly ventilated, and so designed that extraneous

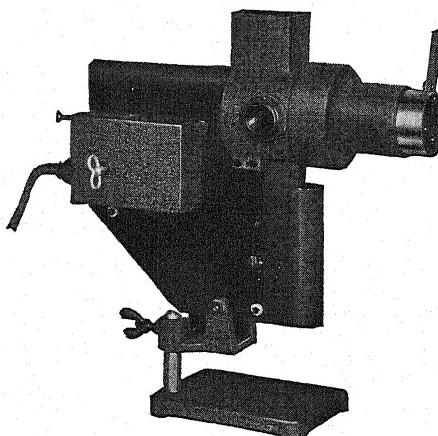


FIG. 53. Bausch & Lomb Automatic Arc Lamp

light in the room is kept to a minimum. (This, of course, is equally true of all types of lamps designed for microscopic work.) Bausch & Lomb's automatic arc lamp is illustrated in Figure 53. The condenser is supplied as an integral part of this lamp. It can be furnished with or without a cooling cell, and arranged to mount on a separate portable base or on standard riders to fit the Bausch & Lomb optical benches.

The Zeiss mechanical-feed arc lamp is shown in Figure 54. This lamp is designed to mount only on the Zeiss triangular optical bench,

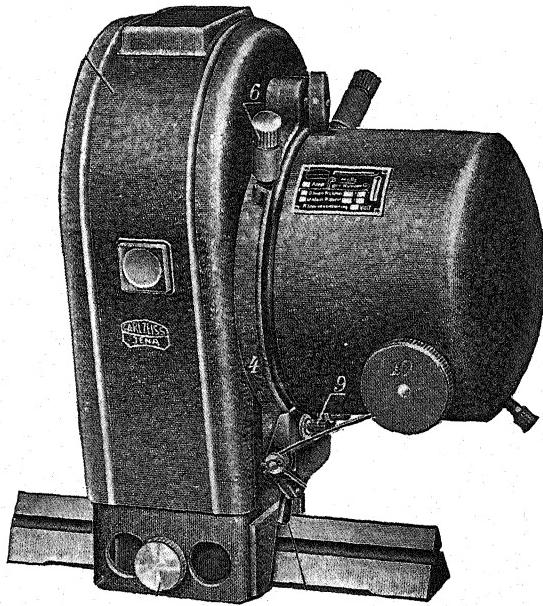


FIG. 54. Zeiss Automatic Arc Lamp

and as all Zeiss condensers, water-cooling cells, etc., mount on the bench on separate riders, the lamp is not equipped with any of these accessories. The Zeiss lamp is unique in one respect — the horizontal carbon is of very small diameter (5 mm.) and is cored. This construction completely eliminates wandering of the arc. The small diameter is compensated for by a more rapid movement of the horizontal carbon and a much greater carbon length, as compared to the larger-diameter vertical carbon.

The arc lamps of other manufacturers follow the general design of one or the other of these two illustrated.

Because arc lamps may have to be used on either direct or alternating current, standard practice is to supply suitable rheostats for use with them. This is the only method of operating arc lamps on direct current; with alternating current, however, a step-down transformer can be employed to advantage. As these are not ordinarily available from the microscope manufacturers, they are often difficult to secure, but are desirable where the heat from the rheostat — which is considerable — is best avoided.

Although the use of arc lamps is essential for some classes of service, for the greater part of ordinary photomicrographic work the light is unnecessarily intense and frequently very objectionable. The devel-

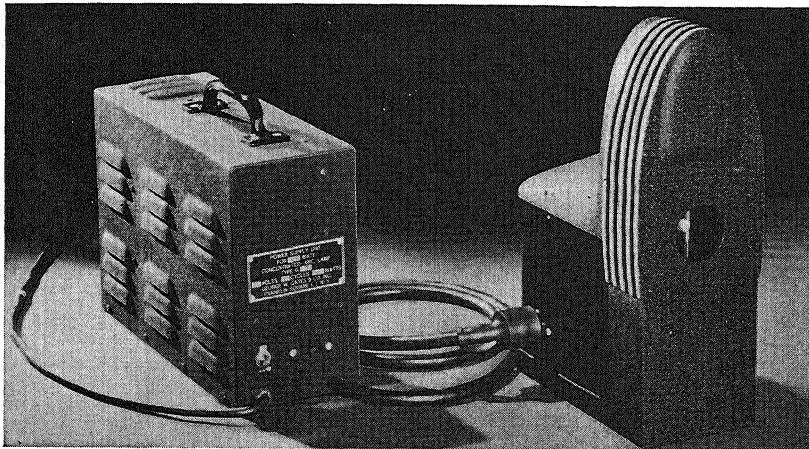


FIG. 55. Bausch & Lomb Zirconium Arc Lamp

opment of other sources of concentrated high-intensity illumination has provided the microscopist with a wide range of lamps, from which he may choose according to the type of work being done.

The lamps available can be grouped in to several general types: zirconium arc lamps and tungsten arc lamps within vacuum bulbs; low-voltage, high-amperage ribbon filament lamps; concentrated-filament projection lamps; photoflood and high-rated (3200°K) lamps; and special types such as mercury-vapor arc lamps, electric sodium lamps, etc. The Bausch & Lomb zirconium lamp, which replaces their now discontinued tungsten arc lamp, is shown in Figure 55. Several other manufacturers also supply zirconium lamps.

Suitable housings are necessary with all of these, but where the type

of base with which lamps are equipped is the same, one type of lamp can usually be substituted for another, when desired. It should be pointed out, however, that lamps in the first two groups require external additional equipment in the form of starting resistances, controls, transformers, etc., to render them operative, so that mere exchange of one type of lamp for another will usually not suffice. Lamps of the vacuum arc type are relatively high priced, especially when the accessory equipment is included. This is also true, to a lesser extent, of low-voltage ribbon-filament lamps.

Concentrated-filament projection bulbs are usually designed to operate directly on 110-volt circuits, so that no extra transformer or rheostat is required. These come, however, in three different basings — the large Mogul base, the standard Edison base, and the bayonet-lock base. The latter is standard for small motion-picture projectors, in various intensities, up to 750 watts; these constitute an ideal light source, although they have not yet been so widely adopted for photomicrographic work as they deserve to be. The same is true of photoflood bulbs. These will undoubtedly soon be put to use in photomicrography, as they constitute one of the least expensive sources of intense illumination. One great advantage both projection bulbs and photoflood lamps possess over other types is the possibility of switching in a resistance to lower the intensity to a point where the preliminary visual work can be done. This also saves current and extends the life of the lamp. For this reason projection and photoflood lamps are included as available modern equipment because, assuming the lamp housing furnished by the manufacturer to be adequate, the particular light source installed within it is readily subject to change, at the will of the photomicrographer; or lamp housings alone may be purchased and equipped with the preferred type of lamp.

Most microscope manufacturers supply tungsten or concentrated-filament lamps, designed with a complete housing which includes efficient condenser systems for providing Köhler illumination, filter holders, and other devices for complete control of the entire illuminating system. The American Optical Company's Advanced Laboratory Illuminator No. 735 employs a multifilament 100-watt, 120-volt lamp as standard equipment, in an air-cooled housing, with a two-lens adjustable condenser and multiple filter holder. It is shown in Figure 56 and in Figure 32, p. 53. For color photography a lamp of 3200°K is substituted for the standard lamp.

The Bausch & Lomb corresponding lamp (Model PR-27), shown

in Figure 33 page 54, employs a 6-volt, 18-ampere ribbon-filament lamp. This requires a transformer for reducing the line voltage to 6 volts.

A radical departure in illuminators, known as the Ortho-Illuminator B, is manufactured by Silge & Kuhne.* It is mounted on a baseboard designed for holding the microscope, and the horizontal axis of the lamp terminates in a 90° reflector which directs the light upward into the microscope condenser. It is illustrated in Figure 57. The lamp is a standard 115-volt, 100-watt bulb of 3200°K, but it does

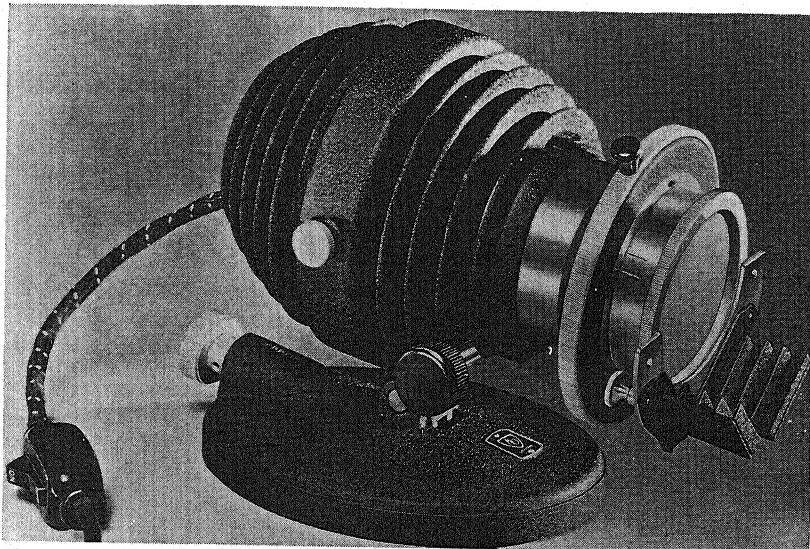


FIG. 56. American Optical Company Advanced Laboratory Microscope Illuminator No. 735C

not serve directly as the light source. Instead it utilizes the method described in the first edition of this book (page 193) as the author's modification of Köhler illumination (see page 218), that is, by means of the interposition of a diffusing disk located in front of the light, which becomes the actual homogeneous light source. In the Ortho-Illuminator B the intensity of the light, its collimation, diameter of field, provision for filters, polarized light, and every condition contributing to perfect illumination by the Köhler method are antici-

* Address, 16th and Carolina Streets, San Francisco 19, Cal. This lamp is now manufactured by the American Optical Co.

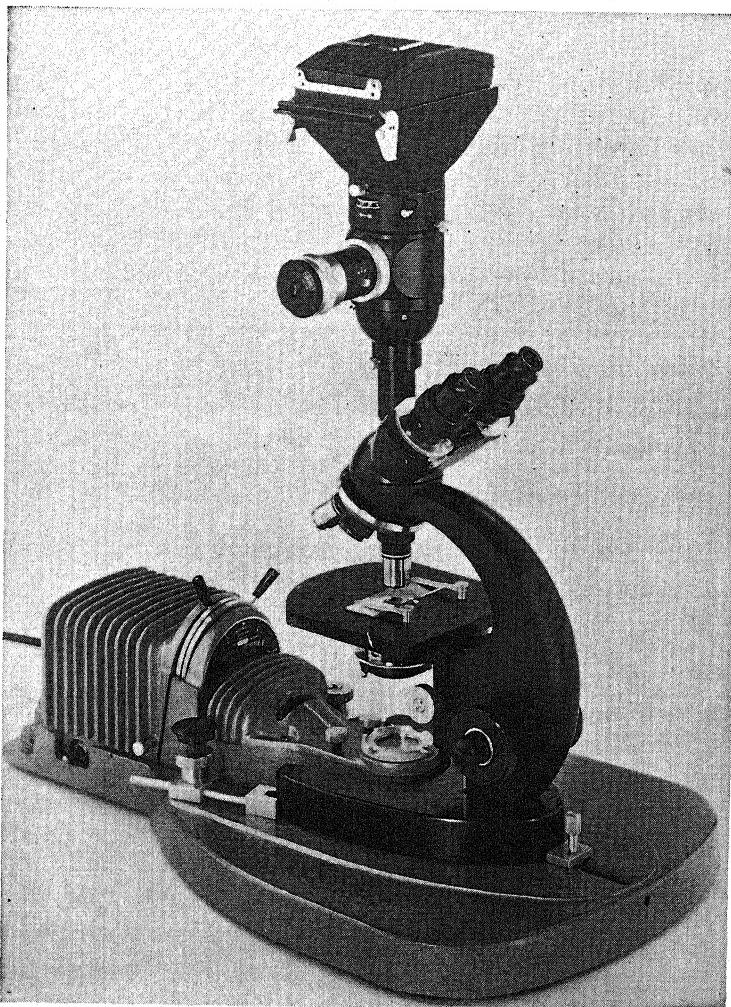


FIG. 57. Silge & Kuhne Ortho-Illuminator B, as Used with a Microscope

pated in this design. The only criticism of this illuminator is that in its present design it is useless for photomicrography with a horizontal camera. A modification to provide for direct projection of the axial beam would be a simple matter. It still, of course, would not be the equal of the 500-watt light source used on the author's camera unless a lamp of this intensity could also be substituted for the present 100-watt bulb.

It should be pointed out in connection with all types of illuminators intended for photomicrography in color that the light source must have a rating of 3200°K for perfect color balance. If the light source is greater or less than this, correcting filters must be employed. This phase of the subject will be discussed at length in Chapter 5 where color photography through the microscope is considered.

In addition to light sources for ordinary photomicrographic purposes, there are others available for specific purposes. Two especially, are of importance — the quartz mercury-vapor arc and the electric sodium lamp. Both of these are rather expensive, but indispensable for certain types of work. Mercury-vapor lamps are available for

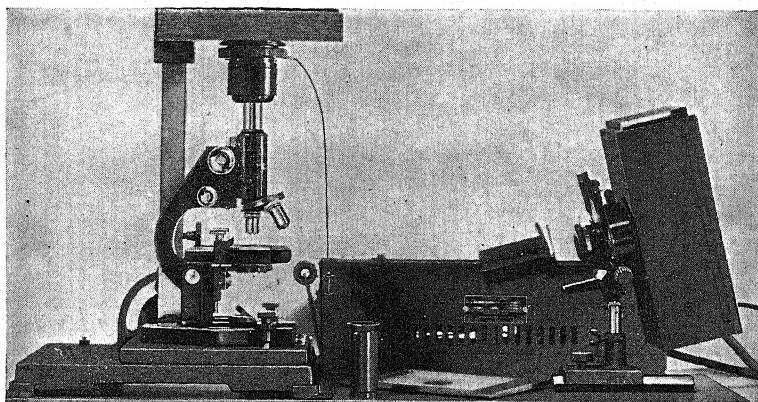


FIG. 58. Bausch & Lomb Quartz Mercury-Vapor Arc Lamp, with an Ultra-Violet Setup

operation on either direct or alternating current. For photomicrographic purposes they are housed in a manner similar to arc lamps, and may be equipped with portable bases or riders for the optical benches, as desired.

Bausch & Lomb's mercury-vapor lamp is illustrated in Figure 58. Mercury-vapor lamps find their chief use in ultra-violet and fluorescence photography, but are also of value when a sharp band in the green region is desired; as the 546-m μ spectral line is so extremely intense, it provides a beautiful monochromatic light source in this region.

The sodium lamp, the Zeiss model of which is illustrated in Figure 59, serves the same purpose as the mercury-vapor lamp in providing a

monochromatic light of great intensity, but in the yellow, through the double D line of the solar spectrum at $580\text{ m}\mu$. No filter is needed with the sodium lamp, but one must always be used with the mercury-vapor lamp, for passing *either* the $365\text{ m}\mu$ or $546\text{ m}\mu$ line, but not both at the same time as would happen if no filter were employed.

All of these light sources find their primary application in the photography of transparent objects, by transmitted illumination, light or dark field. Only the most intense of them are suitable for vertical

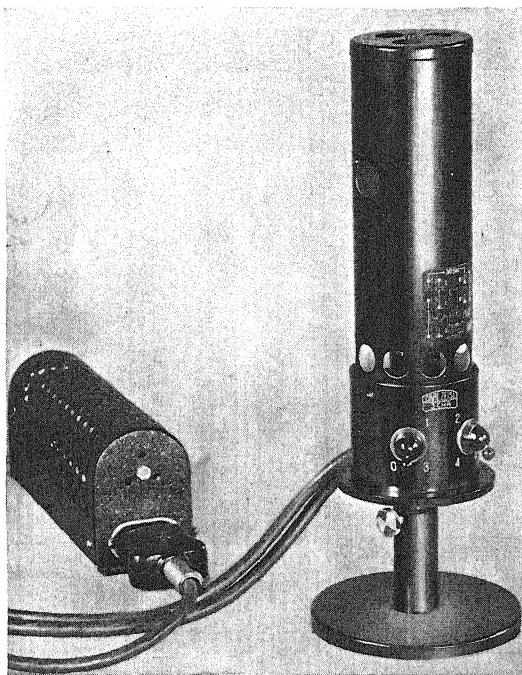


FIG. 59. Zeiss Electric Sodium Lamp

illumination — metallography, etc. The same is true of low-power incident illumination with oblique light. For this purpose an ideal arrangement is an arc lamp with focussing condenser, or other concentrated light source, mounted on a portable floor stand and adjustable as to height — say, from 3 to 5 feet.

Such equipment does not seem to be standard with the microscope manufacturers, but the floor stands are easily procurable and it is a simple matter to mount the arc or other high-intensity lamp, com-

plete in its housing, on the adjustable support. Figure 60 illustrates the general idea.

A very practical illuminating device for low-power photomicrography with either transmitted or incident light, which can also be used for copying and reducing, is the Macro Stage of Zeiss, shown in Figure 30. For opaque work the individual top lamps are used, as desired, the object being supported, with a suitable background, on the flat glass stage. When the object is to be photographed by transmitted light, illumination is effected by lights located under the glass stage, each of which is separately controlled.

Other forms of illuminating devices for low-power work are available from the various manufacturers. The Macro-Ring illuminator and light box of Leitz are shown in Figure 61. Manufacturers are continually bringing out new modifications of this type of equipment, but the general designs remain the same.

Miscellaneous Accessory Equipment

In addition to the cameras, optical benches, and light sources, which constitute the essentials of a photomicrographic outfit, most of the larger manufacturers supply numerous accessories, some of which are almost as important for certain classes of work as the more basic elements. Chief of these is the cool-

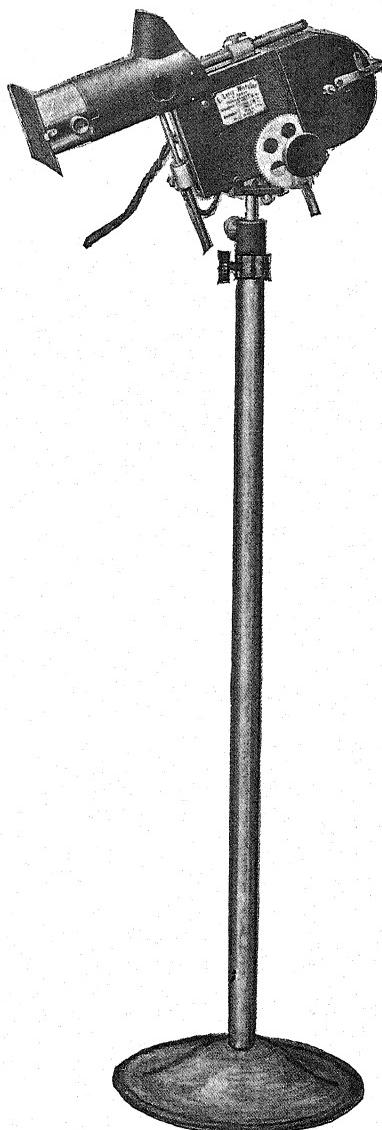


FIG. 60. Arc lamp on floor stand

ing cell, an absolute essential when arc lamps are employed. Some other lamps high in heat rays are also likely to cause damage to lenses or objects unless a cooling cell is inserted in the light train. This is especially true when the so-called Köhler illumination is employed, for high magnifications.

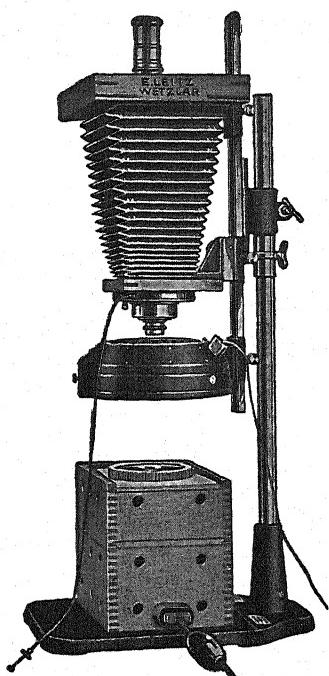


FIG. 61. Leitz Macro Ring Illuminator and Light Box

solutions, if desired. (Information about such solutions is given in Chapter 4.) For most purposes, however, commercial filters are preferable. For this reason, manufacturers provide some means, which can be inserted in the light train, for holding such filters. The filters are available in two forms, the Wratten dyed gelatin films mounted between glass, as supplied by the Eastman Kodak Co., and solid colored glass filters, manufactured by Schott & Gen, Jena, the Corning Glass Co., and others. The two-inch-square size is ample for almost all photomicrographic work, although larger sizes are available

The cooling cell takes the form of a glass container with parallel sides of plane surface glass, capable of holding water or an aqueous solution of salt, alum, or other chemical having a strong absorption for infra-red rays. The Zeiss form, used either singly or in pairs, as required, is shown on its individual dual rider, in Figure 62. This is a very practical design. The body of the container is made of heavy porcelain, the sides of which are ground flat. Plain glass disks are held in place on each side by means of clamping rings; leaking is prevented by the use of rubber packing rings. The cells can be easily taken apart for cleaning or to replace a broken or scratched glass disk. For ultra-violet work the glass disks can be replaced by a pair made from fused clear quartz. These latter, however, are quite expensive.

In addition to their function for cooling purposes, cooling cells can also be utilized for holding aqueous color filter

at a higher price. Neutral tint and heat-absorbing glass filters are supplied by Bausch & Lomb. These are available in different densities.

In special equipment for use in the light train on the large outfits, the Zeiss Co. leads all others, on account of the employment of the one-meter optical bench and the unit design of each piece of apparatus. It must be noted, however, that the advent of the universal Ultraphot has more or less placed such apparatus on the "special" or discontinued list, together with all forms of their larger research photomicrographic models. This special equipment includes special condensing lenses from 7" in diameter down, of various focal lengths; centering condensers; condensers with associated iris diaphragms; large and small iris diaphragms; object-supporting tables; shutters; filter holders; cooling cells of various types; polarizing prism, etc. Each of these is provided with its own individual rider; separate riders are also provided, on which the photomicrographer may mount any special equipment of his own. To anyone working with one of the large outfits, this represents flexibility in the extreme, but at the same time, it assumes a theoretical knowledge capable of setting up and using the various pieces of apparatus, which is often not in evidence.

Another piece of auxiliary apparatus supplied by Zeiss, and which should be made available by every manufacturer of photomicrographic equipment, is the Multipliator Back, shown in Figure 63. This back fits on the camera in place of the regular plateholder, and the latter then mounts on the Multipliator. It enables the plateholder to be shifted, throughout its length, across the centerline of the camera. Then, by means of metal masks, mounted in the camera in front of the Multipliator, having openings of predetermined widths, a whole series of exposures of an identical field can be taken at various exposure times, or with various filters in the light train, for determining ideal exposure conditions. This is a valuable adjunct for eliminating guesswork and making direct comparisons.

It is impractical to attempt to cover all the various pieces of equip-



FIG. 62. Zeiss Cooling Cells on Optical Bench Rider

ment available. Especially is it impossible to do justice to all the auxiliary equipment designed for use with universal outfits such as the Panphot and Ultraphot. When the underlying principles of each type of photomicrographic work are once comprehended, the information in the manufacturer's catalogues is usually ample to guide one as to whether a particular article or piece of apparatus is essential

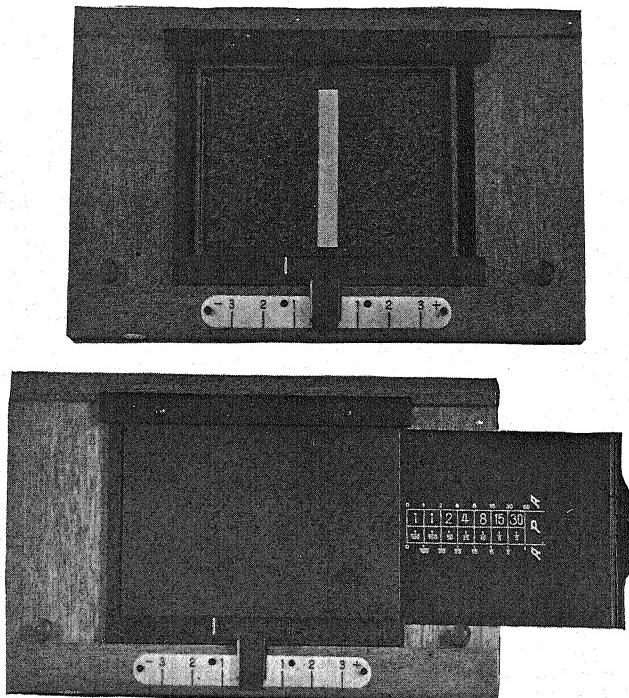


FIG. 63. Zeiss Multiplicator Back

for his peculiar needs. With a general knowledge of these needs before purchase of an outfit, the information given should suffice to enable a proper selection of equipment to be made.

Except for the universal outfits, where the microscope is an integral part of the apparatus, all other photomicrographic cameras assume ownership of a separate microscope which is to be adapted to the outfit. In the majority of cases, the microscope will be used for visual purposes as well.

When the microscope is already on hand and a new one is not con-

templated, it must be used, in spite of any inherent limitations it may possess. When the microscope is to be purchased with the rest of the photomicrographic equipment, there are some points relative to the instrument that must be considered.

Special attention should be given to the following conditions: (1) High-quality work at high magnifications requires a heavy, rigid stand, with the best fine-motion focussing mechanism possible. Fine motions on low-priced stands are often quite coarse for critical work. (2) Low-power work with large camera-type lenses of greater focal length than 60 mm. requires a large tube stand, and ample distance between lens and stage. For this purpose, stands in which the stage is adjustable by rack and pinion are preferable. (3) For high-power work, a slow-motion mechanical stage is essential; otherwise a minute object cannot be centered accurately. *At 2000 diameters, a movement of the object 1/1000 of an inch displaces it 2 inches on the ground glass!* (4) Proper angular orientation of the object with respect to the edges of the picture requires a rotating stage, especially in high-power work. (5) The question of the optics of the microscope should be settled on the basis of the degree of perfection desired in the photomicrograph. Although for visual work achromatic objectives will usually prove adequate, and for ordinary routine photomicrography will also serve (provided they are not forced in their performance), they are far outclassed for photography by the apochromats. These latter should always be used when the highest possible results are desired. For visual work, compensating eyepieces are required when apochromats are used, but these are not ideal for photomicrographic work because of the extreme curvature of the field attendant upon their use.

To produce a flat field with apochromats, Zeiss developed the series of Homal eyepieces, which give beautiful results. These are negative eyepieces, with the eyepoint lying within the lens; hence they cannot be used for visual purposes. More than one of this type of eyepiece is required in order to compensate for the varying degrees of under-correction in apochromatic objectives within the range of low-to-high numerical aperture. A minimum of three is necessary to cover a complete series of objectives. The Homals being of larger diameter than standard oculars, adapters are required for fitting them to the microscope.

Bausch & Lomb put out a similar set of three eyepieces, known as Ultraplanes. These, however, are of the same diameter as standard

eyepieces, and hence do not require special adapters. Their eyepieces are designated as low, medium, and high; these designations refer, not to the eyepiece magnification, but to the group of objectives for which they are computed. The actual magnifications of the eyepieces are $7x$, $8x$, and $9x$, respectively. In this they differ from the Homal set which are all of 20-mm. focus, or $12.5x$. In addition to the standard Homal series, which because of this high magnification do not provide for a sufficiently low range of magnification when used with objectives under $20x$, a fourth low-power Homal (Homal II) with a focal length of 70 mm. (about $3.5x$) is available. It is used primarily with objectives of not over .65 numerical aperture and is calibrated to provide an adjustment in the tube length to yield best results for variations in aperture and magnification. The series has recently been further supplemented with the Homal VI, which provides for an intermediate magnification (with a focal length of 37.5 mm.). Like the Homal II it is used with the low-power objectives, but does not require any adjustment in the tube length. Leitz also supply negative eyepieces for photomicrographic work, similar to the Bausch & Lomb series.

These photographic eyepieces as supplied by Zeiss, Bausch & Lomb, and Leitz are all considerably higher in price than compensating eyepieces; nevertheless they will fully repay all they cost when ideal results are desired.

The substage condenser should also be of a higher order than the ordinary Abbe two- or three-lens types. For most purposes, an aplanatic 1.4 N.A. condenser suffices, but some critical work calls for one that is chromatically as well as spherically corrected.

Finally, for best results, some means should be provided for individually centering each objective with the condenser. It makes no difference, however, whether this is accomplished by centering the condenser or by centering the individual objectives.

Attention to all these details in the selection of a microscope will not be wasted.

Homemade Equipment

The possibility of employing homemade equipment for taking photomicrographs probably suggests itself at some time or other to every owner of a microscope, or at least to those who in addition to the microscope also possess a camera of some description. Naturally the question arises, "If the two could be attached together somehow, could not a picture be taken with the combination?" In many cases only a lack of knowledge as to the optical principles involved, or the proper way to proceed with the mechanical association of the microscope and camera, prevents the putting of the idea into actual practice.

In theory, at least, every owner of a microscope and camera (regardless of the type) does possess the "makings" of a photomicrographic outfit, capable of taking practical pictures, since the availability of a light source (the third essential) of some sort can be assumed. The primary requisite is that they be aligned so that the projected image from the microscope can enter the lens opening of the camera, while, at the same time, all other light is prevented from entering. In the practical application of this requirement several minor factors must be taken into consideration if satisfactory results are to be achieved. Among them are the following:

(1) Some means must be provided for retaining the microscope and camera in proper relationship. This necessitates some simple mechanical mounting for them.

(2) The microscope and camera may be mounted either vertically or horizontally, with equally satisfactory results. The type of camera and relative ease of mounting them together are usually the determining factors, unless the nature of the objects to be photographed necessitates a horizontal stage (e.g., anything mounted in liquid which would not remain in position if the slide were turned on edge).

(3) The preferred arrangement calls for the removal of the camera lens whenever it is feasible to do so, and the use of a ground glass for focussing the image on the plane of the film or plate. Where this cannot be done by means of the camera itself, as in a roll-film camera

(with plate and film-pack cameras it is possible), a focussing tube with a ground glass at one end may be employed. The tube, when mounted over the microscope, should measure the same distance from the eye lens of the eyepiece to the ground glass as that from the eye lens and film, when the camera is in position. Focussing is then accomplished by closing the shutter of the camera, removing the latter, substituting for it the focussing tube (which need not be larger than an inch in diameter), and focussing the image sharply on the ground glass. Then, without disturbing the microscope, the tube is removed, the camera substituted and the exposure made.

(4) Where the camera lens cannot be removed, it is satisfactory to allow it to remain; with cameras which can be equipped with a ground glass in the focal plane, the procedure is not different from that where the lens is removed. But for cameras where the focussing cannot be done in this manner, the focussing must be done by visual observation in the microscope with the camera removed, after which the camera, set at infinity focus, is placed in position and the exposure made. Assuming a normal eye, which in the position of relaxation normally focusses at infinity (thus being, in effect, a camera focussed at infinity when looking through the microscope), it is apparent that when the visual focus is correct, the substitution of any other camera, also focussed for infinity, will not change the condition of focus, and the image should be sharp on the film.

Unfortunately, this method does not always provide ideal results in every case, for two reasons. In the first place, many eyes are not normal, so that when the image is visually sharp it is not actually in focus for infinity. That this is true can be demonstrated by someone with a normal eye observing the image as focussed by one with an abnormal eye, when it will be found that some alteration must be made in the adjustment to produce a sharp image for the normal eye. A second complication arises in the case of fixed-focus cameras, which are not set at the exact focus for infinity, but at a compromise focal position known as the hyperfocal distance, so as to get near-by objects in focus also. The resulting image will not be ideal with such cameras, although it may suffice for many purposes.*

* To provide a personal touch illustrative of this method of taking photomicrographs, the author can relate his induction into the field of photomicrography, when as a lad just out of high school he bought his first microscope before the turn of the century. At this time he was also taking pictures with a fixed-focus $3\frac{1}{2}'' \times 3\frac{1}{2}''$ box camera; it was not surprising, therefore, that the idea of combining the microscope and camera to take pictures soon suggested itself. In his small home town in western

(5) Some practical means, such as that described on page 112, must be provided to assure a light-tight connection between the microscope and camera. If the adjacent surfaces of each are flat and parallel, even a thick felt washer may suffice.

Although the mere juxtaposition of microscope and camera in proper relation will suffice to take pictures, once the thrill of producing even a mediocre micrograph by this means has been experienced, a desire for a more elaborate setup is almost sure to result, since the limitations of so simple a combination will soon become apparent. One will want to go further, with more elaborate apparatus of some sort.

The extensive line of equipment available for photomicrographic purposes, as turned out by various manufacturers, and the consequent great range in price, provide ample accommodation for almost every pocketbook, when one does decide to take up photomicrography in a more pretentious manner. For the ordinary microscopist there is the added thrill of possessing and working with standardized apparatus. It might seem, therefore, that little need should arise for the laborious construction of elaborate homemade equipment, but such an assumption is not wholly verified by facts.

There are two groups of individuals not satisfied with such prosaic means of acquiring a photomicrographic outfit. Strangely enough, from the standpoint of financial rating, some of these are as widely separated as the antipodes, others are scattered in between. Practically all, however, have one thing in common: they are mechanically inclined and derive real pleasure from the building of their own apparatus.

Among the author's friends are perhaps a dozen who are well equipped with the best microscopes that money can buy. They also have workshops, with fine lathes, milling machines, drill presses, and the like, where much of their spare time is spent. It is no wonder, then,

Pennsylvania, without access to any books on the subject and without another individual in the town also possessing a microscope, he was entirely unaware that this had ever been done before. But to think was to act. The procedure was to take the microscope and camera out of doors where bright sunlight was available, focus the microscope visually, and arrange the position of the mirror so that when it was rotated in its pivoted trunnions, direct sunlight would pass across the field of view. The box camera was then balanced on the top of the microscope, the shutter opened and the mirror rotated past the position of illumination by direct sunlight. The effect was one of instantaneous exposure (possibly of the order of 1/50 of a second). The shutter was then closed and the plate developed. By accident, a perfectly timed negative resulted and, considering the quality of the cheap objective with which the microscope was equipped, a fairly sharp picture resulted. Obviously, "what one fool has done, another can do."

that their favorite microscope is not the elaborate product of some world-renowned manufacturer, but one that is homemade in every detail, the lenses only excepted. And it is but natural that some of these who are also interested in photomicrography have constructed the necessary outfit to enable them to take photomicrographs. It does not matter that the ultimate cost has far exceeded that entailed by the purchase of commercial equipment. Often, after the completion of the outfit and the taking of a few photomicrographs to demonstrate that it actually works, its practical value to the maker is about nil; it was not built primarily for use, but for the joy of making it and possessing it afterward, as an example of the maker's mechanical skill.

On the other hand, there are numerous microscopists who have a real longing to take up photography, in a serious way, with the microscope but are financially unable to purchase even the most inexpensive outfit on the market. The fact that there is a limited demand for photomicrographic apparatus of all types, especially as compared to other products more or less in universal use, results in a relatively high cost of manufacture. The cost of marketing is also increased proportionately. These facts explain why simple apparatus, which inherently does not appear to be more than a \$25 value, may bear a price tag of five or six times this amount. They also explain why many individuals, who by pinching and scraping might gradually accumulate a sum of \$25 toward the gratifying of an ambition, either lose all interest when they discover that it would cost them not \$25 but \$125, or else conclude to evade the high costs by constructing the apparatus themselves.

There are hundreds of elaborate homemade photomicrographic outfits in practical use in this country. Possibly there would be many times this number if individuals knew just how to go about designing and building them. It is in the hope of providing such information that the present chapter is included.

Important outcomes of popularizing homemade equipment are the development of greater interest in photomicrography, the training of additional workers, the possibility of publicizing new discoveries in microscopical sciences through the use of photomicrographs, and an ultimate increase in the demand for commercial outfits. All of these are decidedly worth while.

At the outset certain matters should be definitely settled before actually going ahead with the construction of any equipment. The term "equipment" does not imply merely a camera placed at the eye

end of a microscope with a light in front of the mirror, such as already explained. It is true that such combination does embody the use of the three essentials of a photomicrographic outfit, and pictures can be secured in this manner. Very little ingenuity is required to assemble such apparatus; the ingenuity will have to be manifested in the taking of pictures worth showing to anyone as examples of one's ability in things microscopical.

A photomicrographic outfit should be conceived of as a *piece* of equipment, comprising a camera, a mounting base for a microscope, and an illuminating system, all so assembled as to constitute a unit wherein all the parts are definitely related to each other in a fixed manner, and capable of being moved about without interfering with the setup.

The construction of a good photomicrographic outfit requires considerable mechanical ingenuity. If one does not possess such ability, it is better to enlist the aid of a friend who does, even if he knows nothing of the theory of operation. The theory can or should be, in every case, supplied by the microscopist, and he should not undertake any designing or construction work until he understands all that must be accomplished by the apparatus.

The following are some of the important factors governing the ultimate design. Naturally, many of these are identical with those which must be considered in the selection of the proper equipment when it is to be purchased. It is to be assumed that anyone planning to construct homemade equipment along the lines suggested in this chapter will also study carefully the commercial models available, and the particular advantages in each design, before undertaking any construction work.

- (1) What is the maximum size of photomicrograph which might be required?
- (2) What is the general type of work contemplated — transparent, opaque, metallurgical, motion picture, or other?
- (3) Is one type of work to be done exclusively or is it possible that general photomicrographic work of all sorts will be required of the apparatus?
- (4) Will any work be done in which the material to be photographed will be in a liquid medium?
- (5) Are facilities available for constructing many of the parts of metal, or must the entire outfit be made almost exclusively of wood?
- (6) How large can the complete apparatus be, to be used in the space available for it?

- (7) Should it be so designed that it can be easily taken apart for storing, after use?
- (8) What kind of illumination is best suited to the conditions under which it is to be used?
- (9) Is it likely to be used with more than one kind of microscope?
- (10) Are the conditions under which it is expected to be used unusual in any respect?
- (11) Will the work be mostly with low, medium, or high powers?
- (12) Is there any likelihood of the optical bench unit and camera being used for purposes other than photomicrographic — e.g., copying, enlarging, reducing, lantern-slide making, etc.?

Let us analyze the bearing of each of these questions on the final design.

(1) The size of the largest photomicrograph which is to be taken has a direct bearing on the camera required. The size and type of camera, in turn, are tied up with other points for consideration. Although there is now a marked tendency toward the use of small cameras, with a subsequent enlarging of the prints, as in minicam work, many potential photomicrographers would not have facilities available for enlarging. To have this part of the work done commercially, *à la* corner drugstore, is unthinkable. The use of small films will, therefore, in all probability be limited to those who already possess some make of miniature camera and are content merely to mount it on the top of a vertical microscope. Even here it is imperative that some means be available for determining when the image is in focus on the film. Cameras of this type which are equipped to take plateholders and are provided with a ground glass for focussing have a decided advantage in this respect.

In general, in homemade equipment, it can be assumed that a more pretentious camera size will be desired. Usually there is a definite relationship between film or plate size and the bellows extension available. This seriously limits the use of small cameras for photomicrographic purposes. The best commercial type is unquestionably the double-extension view cameras, for here we obtain the maximum possible bellows length. They are not made in sizes smaller than 4 x 5 inches, but, on the other hand, if serious work is to be undertaken, this is the smallest plate size that should be considered. In such a camera, by the use of kits, 3 $\frac{1}{2}$ " x 4 $\frac{1}{2}$ " plates or films can be used for general work, yet the larger size is available should it be required.

This same principle applies to the still larger sizes, 5" x 7", 6½" x 8½", and 8" x 10", and in addition the increased bellows length associated with these sizes is decidedly worth while. View cameras of these sizes, as well as long-bellows cameras of the Poco and Premo styles, are usually available in secondhand condition, at very cheap prices. Even when they are not very serviceable for regular photographic work, because of lack of rigidity, they can still be made to serve in a photomicrographic outfit, provided that the bellows does not leak.

On the whole, wherever possible, preference should be given to the camera with the largest plate size and bellows extension available, for this is the most flexible. It need not be more expensive to operate, because, with the aid of kits, small plates can be used.

(2) The matter of the particular type of work to be done is closely tied up with the degree of flexibility required in the apparatus. Should metallography or low-power opaque photography alone be contemplated, the apparatus best suited for each purpose would not only differ from each other but would be entirely different from that required solely for transparent photomicrography. All three of these could satisfactorily employ either the horizontal or vertical type of camera, although the former is more flexible and also less complicated in the matter of securing and maintaining axial alignment of light for transparent work. On the other hand, whereas transparent work requires the optical bench to be aligned with the optical axis of the microscope, in a horizontal outfit, metallographic photomicrography calls for an optical bench at right angles to the microscope and camera, in order to project light into the vertical illuminator.*

(3) If only one kind of photomicrographic work is contemplated, it usually pays to design the equipment solely with this end in view. But foreknowledge of broader requirements for it will enable modifications to be made in the design to adapt it to any needs. For instance, with ample facilities for producing special apparatus, it is possible, as will be described later, to employ an optical bench planned for transparent work in metallographic work as well. In other words, by knowing all the problems to be met, and all the ways in which the apparatus is to be used, it is possible to design it so that it will be practically universal.

(4) Particles suspended in liquid, or objects which cannot be turned

* This statement must be understood to apply to the use of ordinary microscopes for metallographic purposes. In the modern metallographic outfits, such as illustrated in Figure 140, page 227, the same principle applies, but the optical axis of the objective is at right angles to the illumination train.

on their sides or properly supported in such positions, must be photographed with the microscope in a vertical position. Therefore, for this type of work exclusively, a vertically designed outfit is the simplest solution. Where such work may be only occasional, there are two alternative designs. The first is a combination horizontal-vertical outfit, somewhat like that illustrated in Figure 36. If the outfit is constructed of wood, this is not ideal, although possible. The alternative is a horizontal design in which the camera can be elevated, still in a horizontal position, to above the level of the eyepiece, with the microscope in a vertical position and so located that illumination can be effected with the mirror. Then a right-angle prism, such as that illustrated in Figure 72, can be mounted over the eyepiece to project the image into the camera.

(5) Unquestionably, outfits constructed entirely of metal, along the lines of simple commercial models, are superior in strength to, and less cumbersome than, those made of wood. But few of those most likely to undertake the production of homemade photomicrographic apparatus will have machine-shop facilities available for making metal parts. The alternative lies in the use of wood throughout, or at least for all but the simplest parts. Suggested designs, covering both metal and wood constructions, will be offered later.

(6) Space considerations may or may not be a vital factor. When one has available a large room in which the outfit can be set up, it will usually be found to pay to be generous in every dimension. A large-size camera, a very long bellows, a sturdy table for support, and a long optical bench between microscope and lamp (one meter is an ideal length) will give greater flexibility than can be secured with a small equipment mounting within a couple of square feet. But often one must work in a small apartment room which serves a dozen other purposes as well. In such a case, beggars cannot be choosers; miniature outfits, possibly of the vertical type (as requiring less floor space) must be made to serve. Yet even these can be designed so as to do high-quality work.

(7) One of the corollaries of the small-space outfit is often a need for dismantling the entire outfit after every use, so that it may be stored away in a closet between times. When the dining room or kitchen table, or the bureau in a hall bedroom, happens to be the only place where one can work, it is discouraging, to say the least, and likely to destroy the ardor of any but a born optimist to have to spend a large part of his available time in setting up and taking down the apparatus.

Careful thought given to the design of a simple equipment which can be unpacked and assembled completely in five or ten minutes, all ready for use, then easily dismantled and stored away at the end of an evening session, will materially aid in solving this problem.

(8) In this electrical age, the majority of workers will have current available, so that the problem of light source is limited to the particular type of lamp to be used. But occasions arise where makeshifts must be resorted to. These may be anything from kerosene lamps, gas, battery lights, to daylight, or even direct sunlight. The apparatus must be designed accordingly.

(9) Occasionally a microscopist may use more than one microscope for his work, the second one being a petrographical or metallurgical model. These may not be the same height from the base, when used in the horizontal position; the bases may be of different design; or the relative positions of the base, optical axis, and mirror may be different in the vertical position. If both are to be used interchangeably in a photomicrographic outfit, this must be borne in mind in designing the outfit.

(10) Sometimes a photomicrographic outfit is wanted for a very unusual condition. An illustration of this can be found in the work of the late Warren P. Bentley, whose pictures of snow crystals are known all over the world. His apparatus was homemade and very simply designed for this one specific purpose. It was employed at an open window in a small room in which the temperature was kept below 32° F. The source of illumination was daylight. His setup was so arranged that a picture of a snowflake could be exposed within a few seconds after the flake was placed upon the slide. Use of an outfit in the tropics, on an exploring expedition, etc., might be cited as other examples.

(11) Whether the work contemplated is in the low-, medium-, or high-power regions, or all three, may have a bearing on the design best suited for the purpose. Certainly the higher the magnification, the more rigid must be the construction for the elimination of vibration.

(12) The fact that a horizontal outfit embraces a camera and optical bench in alignment suggests the possibility of utilizing it for purposes other than photomicrographic. If such use is contemplated, the size of the camera is important. An 8" x 10" camera can be employed for making enlargements by loading the plateholder with enlarging paper instead of a plate and providing means on the optical bench for mounting the negative, with illumination from behind.

It is possible that other local conditions or special requirements may be present in addition to these which have been suggested, but enough have been given to illustrate the line of approach to determining the best ultimate design.

So much for generalities; the next step is a consideration of specific design and basic requirements.

One of the first requisites for successful photomicrography is freedom from vibration. This is very essential with high magnification, and correspondingly less so as the magnification is reduced. An appreciation of the way in which vibration affects the photographic image aids in the working out of designs tending to minimize it. It is not the presence of vibration *per se*, introduced through the floor or walls of the room in which the apparatus is used, that is objectionable. It is only such vibration as causes a shift in the image on the plate during the time of exposure, that deteriorates the image. As a rule, the exposures employed in photomicrography are of appreciable length. With instantaneous exposures vibration has no effect. A shift of the image on the plate can be produced only by a relative flexing movement between the microscope and the camera. If the support on which the microscope is mounted also supports the camera and is sufficiently rigid that no vibratory movement can occur which will change the position of the photographic plate with relation to the image being impressed upon it, *vibration of the complete unit as a whole has no effect*. The other extreme, of mounting the camera as a separate and distinct unit from the microscope and optical bench, is the worst possible design unless they are to be used in a room absolutely free from vibration. The moral is, whatever type of photomicrographic equipment is planned, vertical or horizontal, provide a rigid unit support for the camera and microscope. If a discontinuity *must* exist anywhere, so far as vibration is concerned, let it be between the optical bench and microscope.

It must not be thought that there is no need for a fixed relationship between the optical bench and microscope, for this is desirable in order to retain the optical parts in perfect alignment and adjustment with each other. Simple vibration, however, of a magnitude capable of destroying definition when movement is possible between the microscope and camera, will have no effect on the illumination of the specimen, no matter how much movement may result between the illumination system and the microscope, and consequently will not affect the image.

Suggested Basic Designs

With a view to illustrating what can be accomplished with very little expense and yet provide for high-quality results, we suggest a few designs. Let us suppose that a horizontal outfit of generous dimensions is desired, but facilities for working in metal are not available. Wooden construction is the alternative.

Our three fundamental pieces of apparatus, the microscope, camera, and source of light being procured, we are in a position to determine certain essential dimensions — the length of the baseboard, its width, and the height of the optical center. In all probability the size of the camera will be the determining factor in the width of the baseboard. Probably about 8 to 10 inches will accommodate the largest camera likely to be used. Even with a 4" x 5" camera it is preferable to employ a wide base. If, however, size and weight are factors, one can get along with a 6-inch width, especially if the bellows length is not exceptional. The length of the baseboard is governed by the sum of the extreme bellows length, plus the overall length of the microscope in the horizontal position, plus the dimension allowed for the optical bench portion, and in addition, if possible, an extra 10 to 12 inches to allow the camera to slide back from the microscope for preliminary visual work. The space required for the optical bench is that which will carry the lamp, two condensers, filter holder, and a cooling cell, should this be necessary. These can be squeezed into about 18 inches, but twice this space is preferable.

The baseboard should be made from straight-grained wood (almost any kind will serve) free from cracks and well seasoned. Freedom from warping is essential; and a thickness of about $1\frac{1}{4}$ to $1\frac{1}{2}$ inches aids materially. If thinner wood must be used it can be reinforced along the bottom with heavy cleats. On the top side of the baseboard, guide strips, about $\frac{1}{2}$ -inch square, extending the entire length, are screwed along the outside edges. Looked at from the end, the completed baseboard appears as in Figure 64. Care should be taken that the width between the guide strips is the same from end to end so that a block fitted between them will slide the entire distance without binding and without apparent side play. This is all there is to the baseboard, although it should be nicely finished and varnished. Also, a piece of felt cloth can be

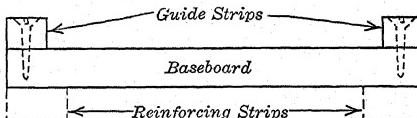


FIG. 64. End View of Baseboard

glued to the bottom if it is to be used on a table where scratching might result. This design should not be used without support under the entire length, as it can be easily affected by vibration.

With this form of baseboard, it is intended that every piece of apparatus used on it be mounted on individual blocks capable of sliding between the guide strips. The height of the mounting blocks for the various apparatus must be such that the optical axis of each will coincide with all the others. All apparatus must also be carefully aligned

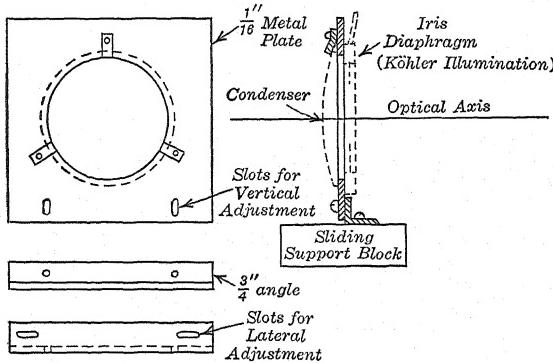


FIG. 65. Simple mounting for condensers

from side to side. Workers with unusual ingenuity can provide means for vertical and horizontal adjustments if they desire.

It is preferable that the camera be mounted on an individual sliding block long enough to accommodate the full bellows extension. The focussing means of the camera itself should provide for changes in the bellows length. With this arrangement, the entire camera can be pushed back out of the way so as to make the necessary preliminary visual examination and select the proper field to be photographed.

After once assembling the camera on its sliding block, it need never be removed. This, however, is not true of the microscope, as it will probably be required much of the time for visual work. For this reason the microscope cannot be screwed to its block, but must be provided with locating means so that the foot will always rest in its exact position. A means of clamping it readily in position must also be provided, as a microscope is not particularly stable in the horizontal position and could be readily dislodged by a jar.

As a light source, where electric current is available, probably one of the simplest is an ordinary photoflood lamp mounted upright in an or-

dinary porcelain socket. The socket is mounted on its wooden slide block so that the center of the lamp globe is in the optical axis. Photoflood lamps are not only inexpensive but have the added advantage of a frosted bulb. A higher-priced alternative, in a clear bulb, is one of the tubular concentrated-filament lamps such as those used with 16 mm. motion-picture projectors. A 500-watt lamp is ample for all work except dark field. If dark-field photomicrographic work is contemplated, an arc lamp is preferable over all others. It should be arranged to mount interchangeably with the lamp used for ordinary work; arc lamps are not so desirable for general transparent illumination, as the

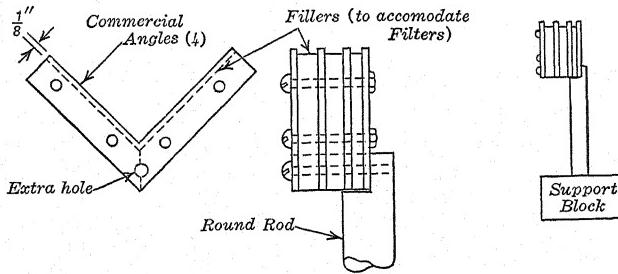


FIG. 66. Filter Holder

exposure is too short for proper control. When more than one light source is to be employed, care should be taken in the original design of the apparatus that the height of the optical axis be sufficiently high to accommodate the taller lamp.

A means of controlling the intensity of photoflood lamps for visual examination purposes will be described later.

Between the lamp mounting and the microscope will be mounted the accessory apparatus of the optical bench. This may include the lamp condenser, secondary centering condenser, a filter holder, and a cooling cell. The condensers, in particular, constitute about the most difficult mounting problem if the construction is to be limited to a wooden design, largely on account of the desirability of centering adjustments. On the other hand, making the condenser mounts of metal offers very little difficulty to the average practical individual. A square piece of $1/16''$ sheet metal with a circular hole in the center, slightly smaller in diameter than the condensers (which should be at least 3 inches in diameter), is the support for the latter. Three small cleats will hold the condenser in place, while two vertical slots in the bottom provide adjustable means for mounting the metal plate to an angle-iron support.

The angle iron, in turn, mounts on the wooden block support. Details of construction are shown in Figure 65. The same design can be worked out in wood also, but naturally the construction is more awkward.

The usual size of filters employed for photomicrographic purposes is two inches square. A holder to accommodate these can be easily constructed along the lines suggested in Figure 66. As different types of filters are of different thicknesses, and as, also, it frequently happens that more than one filter must be employed to provide a definite color band, places for two or three should be provided. These can then accommodate at least two thick filters or one thin and two thick, if three slots are used.

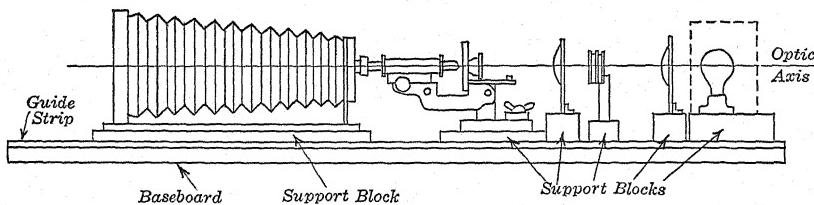


FIG. 67. Design of Horizontal Outfit

If the author's method of illumination, as described on page 218, is employed, no cooling cell is required for a photoflood or 500-watt concentrated-filament lamp. With an arc lamp, however, one must always be used. Preferably the cooling cell should be such a one as those furnished by the manufacturers of photomicrographic equipment, of which there are several types on the market. In lieu of one of these, however, it is possible to employ a large, flat-sided bottle, about one inch to two inches thick. Care must be exercised in the selection of such a bottle; it is usually possible to find one with clear, flat sides. Distortion of the rays from the lamp condenser should be kept to a minimum.

It is desirable to provide a metal shield around the lamp to reduce the extraneous light in the room. This may be easily constructed of galvanized iron. A hole, about $1\frac{1}{2}$ inches in diameter, is cut in the line of the optical axis. In the author's system of illumination, an iris diaphragm, with a corresponding maximum opening, should be mounted on the outside of the lamp housing, over the hole. Also, if a clear lamp

bulb is used, a ground or opal glass should be mounted over the hole, on the inside. This latter can be so made as to be easily removable in case illumination with the clear bulb is required in order to shorten an exposure.

With Köhler illumination, a field diaphragm should be mounted on the lamp condenser supporting plate, as shown by dotted lines in Figure 65. The dimension of the iris diaphragm in this case must be considerably greater than that required when it is used on the lamp housing.

The completed outfit as described is shown in Figure 67. Where metal construction is possible, in place of wood, the primary difference lies in the adaptation of metal parts to the same basic design. The baseboard is replaced by a metal I-beam of the proper length and about $2\frac{1}{2}$ to 3 inches high, depending upon the overall size of the equipment. This I-beam must in turn be mounted on a baseboard, but the latter need not be so heavy as in the case of the all-wood design. The board and I-beam are shown in section in Figure 68. Considerable care must be exercised in the selection of the I-beam. It must be perfectly straight from end to end. In addition, it should also be carefully finished by hand filing or otherwise, on the top and top edges, in order that the apparatus riders may slide freely.

With ingenuity, the riders may be designed to be easily removable, yet capable of being clamped so as to be very rigid. Figures 69A and B show simple designs along this line. It is a good idea not only to mark the positions of each piece of apparatus on the baseboard or optical bench (especially if it is necessary to dismantle the apparatus each time it is used), but also to provide some means of clamping everything solidly to its support as well. In this way all parts of the apparatus, from camera to light source, are rigidly associated at all times. Probably a wooden sub-base for the camera, mounted on the metal riders, will be found to provide the greatest flexibility of operation. It may also be desirable to employ wooden spacing blocks for the microscope and lamp mounting, if there is any material difference in the height of their optical centers.

Where a small amount of money can be spared for a portion of the equipment, a material improvement over either design illustrated would be secured by the purchase of a standard optical bench such as those put out by most of the manufacturers. That of Zeiss, illustrated in Figure 39, is triangular in shape, and one meter long. With such a

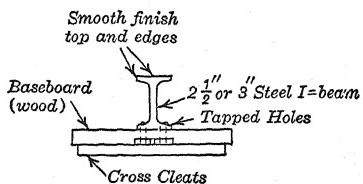


FIG. 68. Base and Optical Bench of Metal Construction

bench, one can then obtain a sole plate for mounting the microscope, and riders carrying all types of accessory apparatus — lamp houses, condensers, cooling cells, filter holders, etc. These items need not be purchased all at once, as makeshifts may be made to suffice until an entire outfit can be gradually accumulated.

These designs in wood and metal will be found ideal so far as a horizontal outfit is concerned. Moreover, they can be readily dismantled for storage in a closet when not in use. Their greatest limitation is that the microscope cannot be used in the vertical position. If both horizontal and vertical work are contemplated a modification can be made in the design, somewhat along the lines suggested in Figure 70. Here the camera portion of the baseboard is separate from that for the microscope and optical bench but is hinged to it so that it can be used either horizontally or elevated to the vertical position. Side braces must be provided for the vertical position to provide for rigidity. The microscope will require a separate base plate for each position, as in one case the optical axis of the illumination apparatus must coincide with that of the microscope in the horizontal position, while in the other it must correspond with the center of the microscope mirror.

Should a strictly vertical camera be desired, the design can still be along the lines shown in Figure 70, but no provision need be made for accommodating the microscope and camera in the horizontal position. This will simplify the construction materially.

An alternative design, which allows the microscope to be used in either the horizontal or vertical position, although the camera is always horizontal, is shown in Figure 71. The camera is elevated to the higher position by means of parallel side pieces, thus kept horizontal through parallelogram motion. An eyepiece right-angle prism must then be supplied to direct the image into the camera.

With these suggestions as to the possible forms which the apparatus might take, enough data have been given to enable anyone reasonably skilled in mechanical construction work to plan the particular type of apparatus best suited to his individual requirements. It should be pointed out, however, that all designs for use with the microscope in the vertical position are inherently more susceptible to vibration than those of the strict horizontal type, and are hence less adapted to very high-power work.

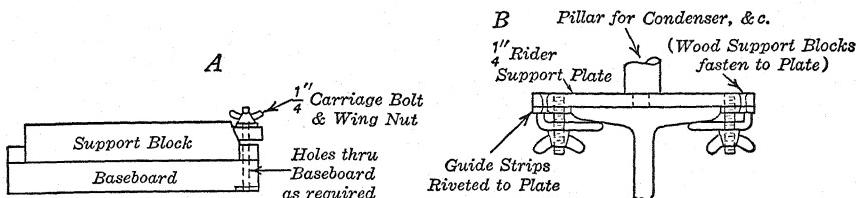


FIG. 69. Clamps for Locking Support Blocks

Provision for Extra-Long Bellows

Complete flexibility of operation calls for a maximum bellows length exceeding that obtainable even with a large-size view camera. When one is forced to use cameras of other types with still shorter bellows, the limitations are still more severe. To this problem there are two solutions worthy of consideration. The first is to employ more than one camera, in tandem; if they are of different sizes, the smaller is placed adjacent to the microscope. It is not difficult to devise an intermediate fitting, preferably of wood, to which the back of the first camera and the front of the second can be attached in some light-tight manner. This intermediate section should be so designed as to provide a central support and at the same time to be capable of adjustable movement for closing the entire bellows length to a short projection distance.

If two cameras are not available, it is not a difficult matter to make either an entirely new bellows of the required length, or an extension to such as may be available. Figure 72A shows a simple method of constructing a bellows. Two layers of material are needed: that to form the outside can be thin rubberized cloth, such as that used for focussing cloth; the other, a firm, dark-colored cloth of the texture of fine muslin. The stiffening strips can be made from thin pressed fiber board about .010" thick, such as that of which filing envelopes are often made. The stiffening strips are first glued in position, as shown in Figure 72B, to the inside of the outer layer, the latter being laid on a flat surface for the purpose. After they are thoroughly dry the inner cloth is glued over the top of the strips, preferably staggering the position of overlap of the two layers so there will not be an undue thickness at this one place. A little ingenuity is required in bringing the two sides together, thus forming the entire structure into a long, rectangular tube. The outside must be glued in place first; when that is sufficiently dry to hold together, the final portion of the inner layer is to be glued down. No attempt should be made to compress

into the final bellows shape until the glue is dry. Then it will be a simple matter to fold the bellows together. It should be compressed completely and held together with pressure until it retains the bellows form. As shown, the design provides for a straight bellows. If a tapered bellows is required, the overall layout is as shown in Figure 73. Each row of stiffening strips, from the large to the small end,

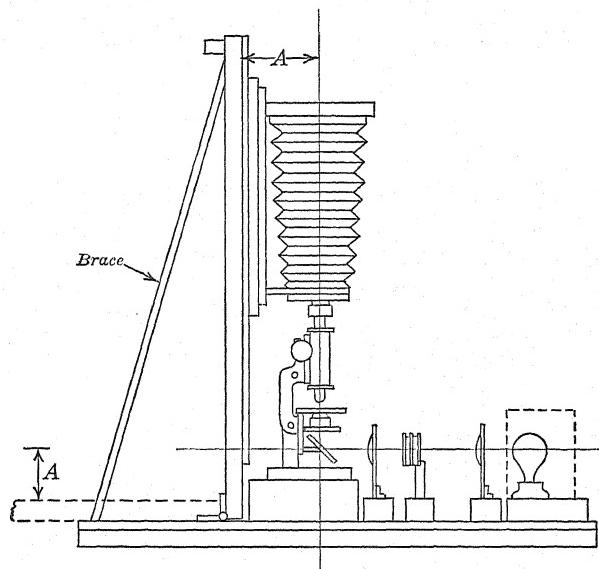


FIG. 70. Combined Vertical-Horizontal Outfit

must be shortened the proper amount over the preceding row to provide for the reduction in size. In either the straight or tapered form, the square end strips at each end provide the box which must mount on the camera front and back.

Connecting Microscope and Camera

Although, as already pointed out, freedom from vibration demands rigidity between the microscope and camera, this must be accomplished *entirely* through the supporting base and *not* through any direct connection with the tube of the microscope. It is imperative that no extraneous light be permitted to enter at this point, yet there must be no physical connection. The reason for this is that after all

focussing is completed on the ground glass, the latter must be removed and the plateholder substituted for it. Then the slide must be withdrawn before the exposure can be made. These operations cannot be performed without subjecting the camera to considerable shaking. If any camera movement can be transferred to the microscope, the focus is likely to be disturbed. This is especially true when high-power oil-immersion lenses are being employed. Connection is therefore effected between the microscope tube and camera by means of what is called a "light trap," a design for which is shown in Figure 74. The two parts of the device can be made of sheet metal by any

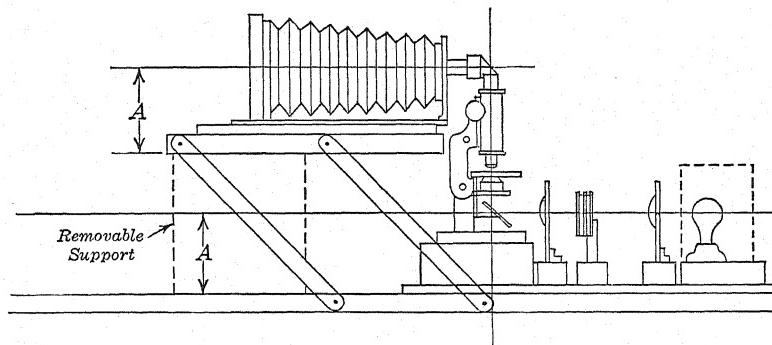


FIG. 71. Combined Horizontal-Vertical Outfit, with Camera always in Horizontal Position

tinsmith or turned out of wood on a wood-turning lathe. The latter construction is obviously more bulky, but equally satisfactory. Whichever form is used, it is important that there be plenty of clearance all around, so that there can be no possibility of touching under any circumstances.

When the microscope is of the large-tube type, such as shown in Figure 51, and provided with a funnel adapted for low-power photomicrographic lenses, connection for the funnel must be of large size, as illustrated in Figure 75. Under this condition, an additional adapter can be made to fit the large ring and provide the light trap for the regular tube.

Simple means should be provided for locking both the front and back of the camera in position so that no change in the focal length can occur during the insertion of the plateholder.

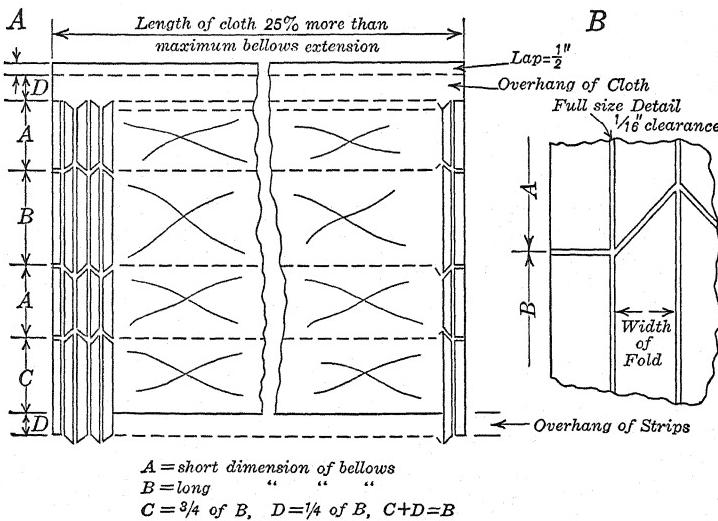


FIG. 72. Construction of Bellows (straight type)

Control of Light Source

The use of a photoflood or concentrated-filament projection bulb as the light source has already been suggested. Either of these will provide adequate illumination for even the highest magnifications, with color filters inserted. When used at full intensity, however, their life is limited (especially that of the photoflood lamps) and current consumption is at a maximum. Experience has shown that the time actually required for focussing on the ground glass and exposing the plate is, on the average, only a small fraction of that spent in examining the slide visually and selecting the proper area to photograph.

For this preliminary visual work, the intensity of any light suitable for taking the picture is far in excess of that which the eye can stand. A common practice is to employ an absorption disk of dark, neutral-tint glass over the eyepiece, or suitable color filters in the path of the illumination beam to reduce the light to the proper intensity for visual work.

There is a much better method which not only adjusts the light to the desired point but saves electricity (somewhat) and materially prolongs the life of the lamp. This is the use of resistances in the lamp circuit, which can be cut in or out as desired. Slide-wire resistances are furnished by the manufacturers of microscopical equipment but are

rather expensive. A good substitute can be very cheaply constructed by anyone, with standard electrical parts costing but a few cents each.

Figure 76 shows circuits employing one, two, and three resistances, with their accompanying switches. The resistances are heater units, such as those used in small portable electric heaters of the reflector type. They are equipped with standard Edison bases and hence mount in ordinary porcelain base sockets. The switches are of the regular toggle snap switch type.

When a single resistance is used, but one reduced intensity is provided and it may be necessary to modify the winding of the resistance to obtain the best illumination for visual work. Lamps of various wattages could also be used for resistance, but as they must be of high wattage ratings, they are more expensive than the resistor units and may also be objectionable because of the intensity of their glow, unless mounted in a closed box. With three resistance units and three switches, five combinations are possible, which should provide ample variation to meet any condition.

If photography with an arc lamp as the source of illumination is necessary, an auxiliary lamp for visual work is often desirable. This can be a low-wattage lamp mounted temporarily in front of the arc lamp and used only for visual work in selecting a suitable field. One need not care whether the setup is ideal so far as critical illumination is concerned; what is most desired is a quick change-over to the arc lamp when all is ready for transferring the scene of operations to the ground-glass focussing screen. The use of the auxiliary lamp not only provides the necessary reduction in intensity of illumination but saves materially on carbons and current.

Condensing Lenses Required

When homemade construction is undertaken it is sometimes a difficult matter to find lenses suitable for the light-source condenser, as this requires a fairly large lens of short focal length. The desirable condition is a condenser which will entirely fill the largest aperture of the substage condenser, when employing the so-called Köhler method of illumination. Three factors determine whether or not this condi-

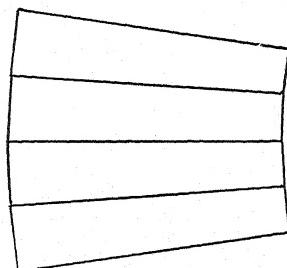


FIG. 73. Shape of Bellows Cloth (tapered type)

tion is met — the diameter of the condenser, its focal length, and the distance between the light source and the microscope. As the focal length is increased, the diameter must also be increased, and *vice versa*. Increasing the distance between the light source and the microscope necessitates the use of a larger-diameter condenser or one with a shorter focal length.

When the author's method of securing critical illumination, described on page 218, is employed, the characteristics of the light-

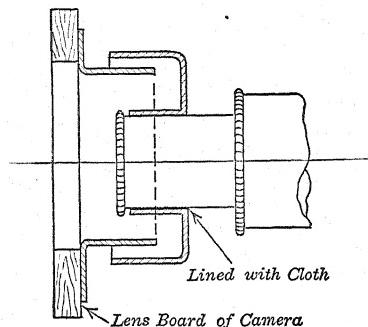


FIG. 74. Light Trap for Connection between Microscope and Camera

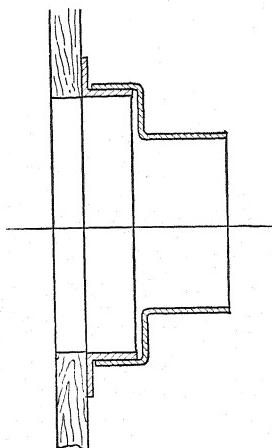


FIG. 75. Adapter for Use with a Low-Power, Wide-Tube Funnel.

source condenser are not so critical since the principal requirement is that it be larger than the back lens of the substage condenser, by a fair margin. This is because parallel, instead of converging rays, are involved, and the distance from the light to the microscope is not a factor.

In general, for average conditions, a condenser with a minimum diameter of at least three inches and a focus of five inches or less, is a good combination. It can be either a double or plano-convex lens. The compound unit of two plano-convex lenses such as those used in stereopticons is ideal. These can often be picked up, mounted in a unit cell, from dealers in photographic supplies. Lenses of almost any size and focal length can be purchased unmounted, direct from optical manufacturers.*

In an emergency a 3-inch reading glass can be used and if the back

* Bausch & Lomb Optical Co., Rochester, N. Y.; American Optical Company, Buffalo 15, N. Y.

lens of the substage condenser is not filled, a duplicate second lens can be mounted directly in front of the first, the resulting combination providing a sufficiently short focus. A reading glass is especially servicable as a secondary centering condenser, located near the micro-

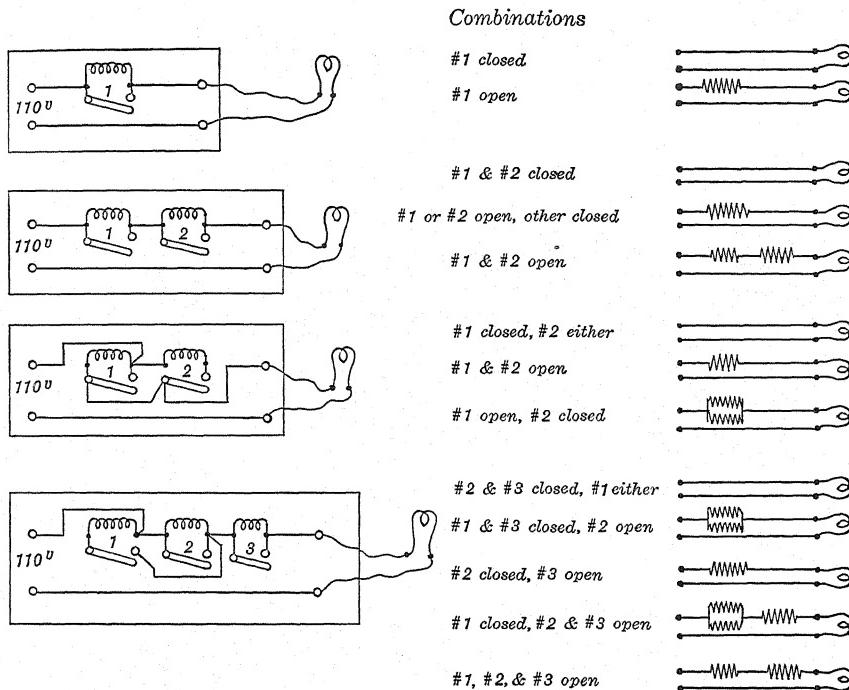


FIG. 76. Control of Illumination Intensity with Cut-in Resistances

scope, to concentrate and center the beam into the substage condenser, but preference should be given here to the longest procurable focal length.

Means for Controlling the Exposure

The reader may have noticed that so far no mention has been made of the location of a shutter for timing exposures. That there is need of some device for accomplishing this is evident. No light may be allowed to reach the plate while the plateholder slide is being withdrawn or replaced, since shaking of the camera is inevitable at such times. Under some conditions it may suffice to turn on and off the

lamp switch, but this is not always wise, as the time of heating up and cooling down, and the difference in the color value of the light before it reaches a stable temperature, may influence the result.

A shutter of some sort is indicated. It does not follow, however, that an elaborate camera shutter is required. One of fairly large diameter is rather expensive, and if it is to be accurate in the timing of fractional parts of a second, only those of the highest quality are satis-

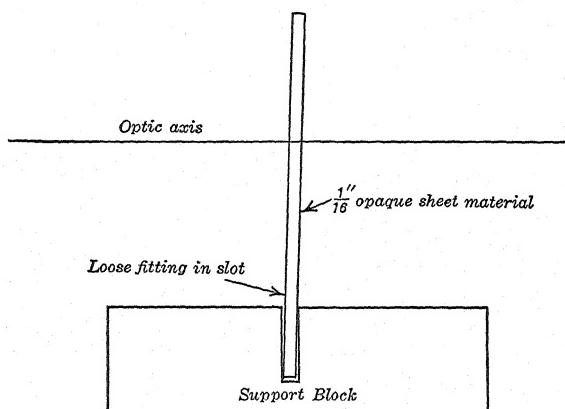


FIG. 77. Shutter Substitute

factory. Short exposures — i.e., under one second — are not ideal for microscopical work.

In many respects a simple sheet of opaque material, such as hard rubber, micarta, transite board, or metal, interposed in the path of the light is equal or superior to a shutter. Either a separate block on the optical bench portion of the baseboard, provided with a narrow slot, such as shown in Figure 77, or a slot in one of the other mounting blocks, can be used to support the light shield. For use it is merely removed by hand for the desired time interval and then replaced. Short exposures, down to one-quarter of a second, can be made by this means if necessary.

Illumination System for Metallography

Where the photomicrographic outfit is required primarily for metallographic work, the baseboard should be L-shaped instead of straight, or a vertical microscope and camera employed so that the illumina-

tion rays may enter the vertical illuminator at the side. For a universal outfit a T-shaped construction with two optical benches at right angles to each other offers a satisfactory solution. When this is impossible because of lack of room, or because metallographic work is contemplated only at rare intervals, a special illumination system can be made to serve. To construct it, however, requires machine-shop facilities and it must be accurately designed and built. Figure 78 shows the nature of this device and the principle of its operation.

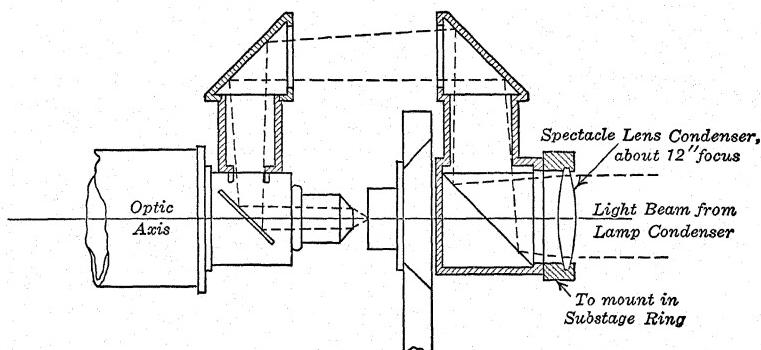


FIG. 78. Metallographic Illuminator for Straight Horizontal Outfit

It is composed of two units: one mounts in the substage ring which ordinarily supports the condenser, the other mounts in the side opening of the vertical illuminator. The former contains a positive lens of fairly long focus and two right-angle total reflecting prisms. The extension tube mounted on the vertical illuminator carries a third right-angle prism. In such a device, combined with the reflecting glass of the vertical illuminator, the light makes four right-angle bends, passing around the microscope stage in so doing, and is finally projected onto the specimen in a direction exactly the opposite to that from which it left the light source.

One advantage of such a method of illumination is that it can be employed with any ordinary microscope and does not require the stage to be movable, as in the ordinary metallurgical microscope.

The recent development of illuminating tubes made from transparent Lucite makes possible the substitution of an S-bend of this material in place of the double prisms mounted in the substage.

Supporting Table for the Photomicrographic Outfit

Where the baseboard must be placed upon an ordinary table for use, it will be found valuable to employ sponge rubber pads (such as the well-known kneeling pads) under it to eliminate vibration; three are ordinarily sufficient.

When a special table can be constructed for it, the legs should rest upon blocks at least six inches square. Between these and the floor can be inserted sponge rubber pads, which will reduce vibrations to a minimum.

The suggestions given should be adequate to enable anyone to design and construct just the kind of apparatus most suited to his peculiar needs. From this point on, it is largely a question of learning how to use the outfit. That knowledge, it is hoped, may be derived, in part at least, from other chapters in this book.

The Technique of Photomicrography

Broadly speaking, the technique of photomicrography can be divided into two distinct parts — the purely photographic, manipulative portion of the work, and the operation of the photomicrographic apparatus to produce a magnified image that will correctly portray the appearance of the object as seen under the microscope.

The first consists of the darkroom processes — developing the negative, making the finished prints, all the knowledge and practices that are the skills of any good professional or amateur photographer. The technique with which we are concerned here is largely limited to the proper operation of the apparatus, up to and including the exposure of the negative, so as to produce the desired result.

This discussion must therefore assume previous knowledge on the part of the photomicrographic tyro, ample to cover the darkroom technique. Only occasionally do the two phases of the subject overlap to the extent that some mention of strictly photographic matters must be made in connection with the making of the negative. Should the beginner not understand such references, because of ignorance of photographic practice, he should consult Chapter 9, which covers the basic principles of this part of the work.

Naturally the amateur or professional photographer is already equipped with knowledge which should enable him to grasp quickly the remaining principles of photomicrographic technique. Should he, at the same time, be familiar with the use of the microscope for visual purposes, he is still farther along. Unfortunately, some in this category rashly conclude that they know all about the combined subjects and proceed to the actual work — with inevitably mediocre results. They entirely overlook the fact that success comes from knowing how to co-ordinate properly the two parts of their knowledge. Moreover, as frequently happens, they may grossly exaggerate their knowledge of proper microscopical technique.

There is much more to the taking of good photomicrographs than merely allowing the microscopical image to be projected onto a sen-

sitized emulsion for the time required to produce a negative of proper density. The further one advances in photomicrographic experience, the more he realizes this.

In presenting practical information on photomicrography, although it is desirable to assume on the part of the reader a working knowledge of simple photographic processes, it may nevertheless be of material help to those taking up a combination of the parts of the work for the first time, to assume their complete ignorance of the general subject, and a need for starting at the very bottom. Obviously those advanced in photomicrographic experience will be familiar with much that is said, and may find only here and there information useful for improving their technique.

Preliminary Considerations

If one decides to take up photomicrography, either as a hobby or commercially in connection with some other line of work, the first consideration must be a study of all the factors involved. The extent of the types of work to be done; the amount of money which can be invested in equipment; the room where the apparatus will be located; the darkroom facilities; the equipment best suited to meet all the conditions — all these must be settled and the apparatus secured that will be best suited to the job.

(a) The Workroom

Some conditions must be satisfied in the room where the work is to be done. The first is freedom from vibration to as great a degree as possible. Vibrations result from so many causes, both external and internal, that some may not be suspected until after the apparatus is set up. In private homes, frame structures, etc., they may be caused by people walking on stairs and over floors, closing doors, and the like. These can be classed as internal causes, and are usually subject to control. A more serious internal cause occurs when the floor of the room where the apparatus is set up or the table upon which it rests is not solid, so that even slight movement of the operator, while the exposure is being made, results in vibration and a consequent fuzzy picture. When it is known that such conditions exist, it is usually possible to overcome them.

More serious vibration problems are those created externally, which are unavoidable and can only be mitigated by damping the photomi-

crographic equipment until the vibrations do not reach the ground glass of the camera. The causes of vibrations of this nature include hard windstorms, trucks on near-by streets, trains on building slidings, subway and elevated trains, the operation of steam hammers and other heavy machinery in near-by factories, etc. Here and there the earth's strata are such that vibrations from a heavy forging hammer may travel for a half mile or more in a particular direction, to the extent that dishes will be vibrated off a shelf or other startling evidences of the forces operating will appear, although in another direction the effect will hardly be noticed a few hundred feet away. For this reason it is a good idea, in setting up a photomicrographic laboratory in a big plant, to assure freedom from such trouble before going to the expense of equipping the room chosen for the photomicrographic work.

Although basements and ground floors, especially when cemented, usually provide the best locations, these are not necessarily the only solutions to the problem of vibration, nor is freedom from vibration the only requisite.

The room should be as free from dust as possible, and also light and airy, yet capable of being darkened by suitable curtains during the taking of a picture and its preliminary examination on the ground glass. Freedom from chemical fumes, especially acids, is another important consideration. Under no circumstances should the apparatus be set up in a room where chemical work is being done, for deterioration will render the apparatus worthless within a very short time.

Proximity to the darkroom is also a desirable feature. The darkroom itself is a prerequisite, for one cannot expect to carry on general photomicrographic work without doing his own developing and printing. Of course amateurs, taking up the work as a hobby, can improvise a darkroom in the kitchen, bathroom, or a closet and can plan to do their photographic work in any convenient room, dismantling and storing the apparatus if necessary when they are through for the evening. All these conditions, however, should be considered beforehand and a course of procedure determined accordingly.

If, as we present the various problems confronting the photomicrographer, we seem to take for granted conditions and equipment far beyond the capacity of the humble beginner, he should not consider himself ruled out. It is rather merely that, by the assumption of ideal conditions, the entire subject may be covered in the manner best suited to giving the maximum information. This policy, therefore, will be followed throughout this chapter.

(b) Setting Up the Apparatus

The photomicrographic outfit having been determined upon, purchased, and delivered, the first steps are to get it properly set up and to become thoroughly familiar with each part — its name, function, and mode of operation. To achieve this, it may be necessary to make liberal use of the manufacturer's catalogues and, if any, their instruction sheet on the specific outfit. With the simpler equipment there may not be much auxiliary apparatus.

Attached cameras of the 35-mm. size or larger require little preliminary setting up except placing them on the microscope tube. With these, most of the preliminary experimentation will be limited to the manipulation of the light source adopted for use with the outfit, and the securing of proper illumination. It must not be overlooked that with these simple outfits the same fundamental principles of illumination operate as with the most elaborate equipment.

In methods of operation, these minicam outfits are so radically different from even the simplest of the conventional photomicrographic cameras that users will do well to master the special techniques covering them published by Leitz and Zeiss. A thorough treatise on the Leica in photomicrography is contained in the chapters on this subject in the *Leica Manual and Data Book*.*

With detailed instructions for setting up and operating these outfits, one is adequately prepared to start work. Later on it will be found that much of the matter of this chapter will be useful even in the operation of these small outfits.

Practically all the more elaborate camera outfits as now supplied include means for adapting 35-mm. cameras to them. As these are all more or less individually designed, instructions provided by the manufacturers should be followed. Integral with these is of necessity a split-beam eyepiece or other device for viewing the object and focussing it on the film. In this connection the numerous makes of minicams now on the market must be considered as to their suitability for use with these outfits. Not all are satisfactory because of mechanical design and other limitations.

Recent advances in the design of universal cameras, almost invariably of the vertical type, together with the large amount of ac-

* Chapter 11, Copying and Close-ups, and Chapter 14, Medical Photography, in H. M. Lester and W. D. Morgan, *The Leica Manual and Data Book*, 13th edition, Morgan & Morgan, Inc., Publishers, New York, \$6.

cessory apparatus and equipment available for making them universal, obviate much of the need for instructions for setting them up, other than the instructions supplied by the manufacturers. With these, the only matters requiring careful attention are the aligning of the microscope with the optic axis of the camera and fixing it permanently in position with the adjustable means provided; then aligning the light source, either to project on the center of the mirror, or in case of illuminators designed to project the light vertically into the condenser, to ensure that these are in line.

With the large self-contained models where even the microscope is an integral part of the apparatus, practically no setting up is required. The instructions furnished with the outfits need only be followed for the various ways in which the equipment can function. However, all these universal and self-contained models operate more or less automatically along principles applying to photomicrography of earlier years, when each piece of apparatus had to be assembled independently — principles to which rigid adherence must be maintained to secure ideal results.

There are a great many pieces of older equipment that are now in use and probably will continue to be in use for some time to come. The majority of these are of regular bellows design, either of the vertical or horizontal types, as well as the combined horizontal-vertical models constituting the research models of former days. The latter provide for more universal use than even the finest models now extant. The only objections to them were their size and the space they occupied, and the necessity of being an expert microscopist to operate them. (One of the motives for present designs is to produce apparatus that almost anyone can operate.)

In view of this, it is important that information covering of older vintage cameras be available for those who must use them. Therefore some of the steps in setting up and operating will apply only to this general class of apparatus. Some of the instructions offered will, nevertheless, be of value in getting the most out of even the latest designs in photomicrographic equipment, which are many and extremely varied in appearance, if not in practical operation.

In the more elaborate outfits, some of the preliminary points to be noted are: The method of extending and shortening the camera bellows length; the nature of the light-trap connection between the camera and microscope; and the manner in which the camera is moved out of the way to allow visual examination. It should be noted that in

bringing the camera light-trap to the microscope, it must always be so located as to exclude completely all room light, and yet there should be no physical connection between camera and microscope. The reason for this is to obviate possibility of jarring the microscope out of focus when the plateholder is being inserted and the slide withdrawn. Some shaking of the camera is bound to occur, but this does no harm if the microscope is undisturbed.

Several types of plateholders are in use on photomicrographic cameras. It is necessary to become thoroughly familiar with their operation, especially the manner of loading them, for in many classes of work the loading must be done in absolute darkness. Under most other conditions the faint red or green safelight allowable gives very little light. On the smaller and less expensive cameras the plateholders may be single, and of metal. Some cameras employ standard wooden

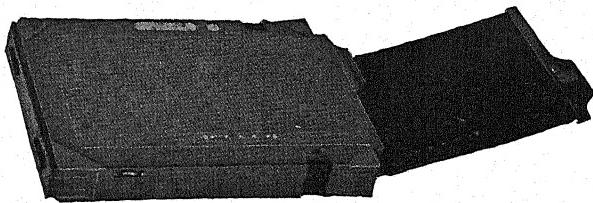


FIG. 79. Zeiss Booktype Plate Holder

double holders. The most elaborate holders, used on the large older models of Bausch & Lomb and Zeiss, are of the book type, hinged so as to open in the center. The book type plateholder of the latter is shown in Figure 79. One can practice in daylight, using a suitable piece of glass or an old negative, loading and unloading until it can be done in complete darkness. Especially it should be remembered that the emulsion side always faces the microscope. Thus while the regular type of holder must have the emulsion *up* (i.e., toward the outside) before the slide is put in place, the book type holders, which load from the center, must have the emulsion side *down*. If cut film is used, it is inserted in the cut-film holder emulsion side up, and then put into the plateholder just as if it were a glass plate. With book type holders, no cut-film holder is necessary; the film is laid in place, emulsion side down, and a glass plate of the same size is laid on top of it, to take the thrust of the retaining springs off the film, yet holding it firmly in place.

The operation of the lamp should be studied next. This is especially desirable with a hand or automatic feed arc lamp, its various adjustments, centering means, etc. The effect of moving the lamp condenser in and out, or forward and backward on the optical bench, should be observed. Later on we shall see how proper illumination may be secured through manipulation of the condenser. The effect of opening and closing iris diaphragms should be noted. If the type of lamp to be used calls for the use of a cooling cell, this should be filled with distilled water or a filtered solution of alum, and put in place on the optical bench at this time.

Fully ninety per cent of all photomicrographic work is done with transmitted light; the most notable exceptions are low- and medium-power work on opaque objects, usually with oblique incident light, and metallographic work. The former will be considered later in this chapter, and metallography will be covered in Chapter 5. At present we can concentrate wholly upon the problems of transmitted-light photography.

(c) *Securing Optical Alignment*

The primary requisite in this type of work is the securing of correct optical alignment of all parts from the center of the light source to the center of the camera ground glass. One of the first things which should be done (unless already provided by the camera manufacturer is to mark a small diagonal cross in the center of the ground glass, by means of a soft lead pencil. This will be at the crossing of the lines from the opposite corners. At the same time, if the plate size of the camera is large, the position of smaller plates, when used in plateholder kits, should be marked. The usual sizes of plates smaller than 8" x 10" are $6\frac{1}{2}'' \times 8\frac{1}{2}''$, 5" x 7", 4" x 5" and $3\frac{1}{4}'' \times 4\frac{1}{4}''$. The mark for this latter size will also serve for lantern slide plates, which are $3\frac{1}{4}'' \times 4''$.

Two conditions are possible in alignment, depending upon whether the outfit is of the vertical or horizontal type. Correct alignment is much easier to secure with a horizontal camera and microscope than with the vertical setup, one good reason why the horizontal camera is preferable wherever it can be used. Understanding the procedure to be followed where the optic axis is straight, from end to end, helps materially in accomplishing the alignment of a vertical outfit.

Let us therefore assume that we are setting up a large horizontal outfit, and follow through the procedure step by step. Obviously one point on the optic axis is definitely fixed; it is the center of the camera ground glass. This therefore constitutes one end of the axis. The camera ground glass should be placed at the distal end of its adjustable movement, i.e., the bellows fully extended. Next the lamp should be set up at the opposite end. The extent to which the correct position of the light source can be established on the optic axis at this time depends on several factors. The lamp may not be adjustable; its condenser height may be fixed, although the light itself is adjustable; the position of the lamp when properly aligned may be indicated by the manufacturer. Or, finally, the position of all parts may be adjustable so that their final positions cannot be set until after the microscope is in place. In this case only an approximation can be made at this time. If it is possible to adjust the light, either by means of the position of its condenser, or by setting to the manufacturer's marks, it should be done at once. At this stage, if the lamp be lighted and its condenser focussed along the optic axis, it should project a circle of light symmetrically around the cross marks on the focussing glass. If it fails to do so, whatever adjustments are provided must be manipulated until the desired result is obtained.

We are then ready to insert the microscope into the system. This is ordinarily located on a sole-plate, with means for holding it rigidly in place and other means for relocating it in the same position, after it has been removed. As a rule, adjustments are provided for bringing the optical axis of the microscope accurately in line with the axis of the photomicrographic outfit. The microscope is bent over into its horizontal position and the mirror removed. Objective, eyepiece, and substage condenser are omitted, but the iris diaphragm of the substage is allowed to remain. In case a pinhole eyepiece cap is not available, a substitute disk can be made of opaque cardboard which will fit snugly into the eyepiece tube. This should have a pinhole in the exact center. With the substage diaphragm closed to a pinhole and the pinhole eyepiece cap in place, the position of the microscope on its sole-plate must now be adjusted until light from the light source, passing through its condenser, then through the two pinhole diaphragms of the microscope, registers a small disk of light on the cross lines of the focussing screen. Not until this is accomplished is the alignment perfect. Right here is the heart of ideal photomicrography,

and an explanation of much of the mediocre work one often sees produced on the most elaborate of outfits.

When this condition is met, it can be assumed that the quality of the optical parts of the microscope (as to alignment) on all instruments turned out by reputable manufacturers, is such that final alignment is assured, except for the minute adjustment which must be made to align each objective with the condenser.

Some illumination systems require a second condenser in the light train, ahead of the substage condenser. It is often called (especially by Zeiss) a centering condenser. It should always be adjustable as to up and down and side to side position, when used, but should not be in the light train when making the preliminary alignment, nor should it be employed to correct any but very minor faults in alignment.

(d) Alignment of Vertical Outfits

Alignment of the parts of a vertical outfit does not differ in theory from that of the horizontal type. The complication making this somewhat harder is that there are two optic axes at an angle to each other. The point common to both is the center of the microscope mirror, which must be employed to alter the direction of the light rays. Unfortunately, several conditions frequently present in the design of the mirror itself complicate the situation and make the problem of alignment one of "cut and try." Then again, the angle between the two axes may be a right angle, or, as is the present common practice, an acute angle, differing with various equipments.

The ideal design of mirror — considering the plane side only, the one that should always be used — would have the exact center of the silvered surface always lie in the optic axis of the microscope, regardless of its angular position. A further desirable feature for photomicrography would be the employment of front surface mirrors. This would not only eliminate the double reflection present in ordinary mirrors but enable alignment to be accomplished easily by the use of a pinhole diaphragm cap which could be dropped over the entire mirror. Figure 80 illustrates the ideal condition, which can only be approximated with present apparatus, and also various situations arising in actual service. It is to be hoped that some enterprising manufacturer will design a suitable mirror and mirror arm, with supplemental pinhole diaphragm, along the lines suggested.

Another objectionable feature present in ordinary microscope mirrors is the ease with which they can be moved. Even after considerable time has been spent in securing alignment, accidental contact

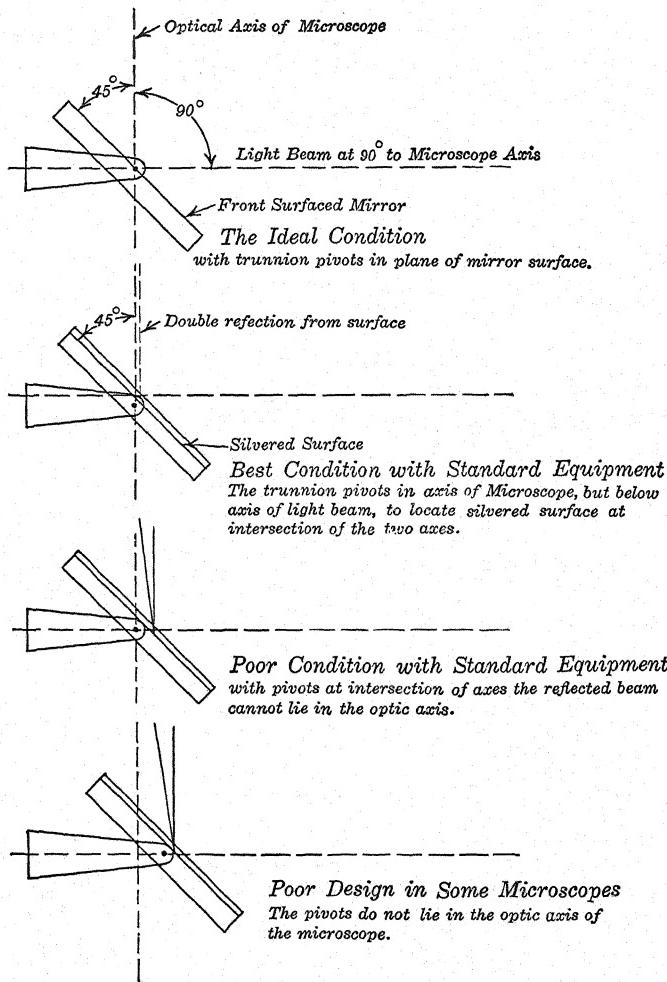


FIG. 80. Operation of Mirror with Microscope in Vertical Position

with the mirror will throw it completely out and all the work must be done over again. To obviate this trouble, some manufacturers make a fixed mirror, adjustable only by screws.

The most practical procedure in aligning a vertical outfit is to employ an opaque but light-colored card diaphragm over the mirror, the hole being around $\frac{1}{4}$ " to $\frac{3}{8}$ " in diameter. The light can be centered on the hole, and the mirror adjusted until the light passes through the two pinhole diaphragms of the microscope, to the center of the focussing screen.

Some workers employ a total reflecting right-angle prism instead of the mirror, but this can be done only when the two optic axes are substantially at right angles.

(e) Preliminary Testing of Equipment

Having secured primary optical alignment, the next step is to assemble an objective (a 10x [16 mm.] is ideal for the first experiments), a low-power eyepiece, and the substage condenser on the microscope. The camera should be moved out of the way, in the manner provided, so that preliminary visual work may be done. A prepared object, preferably a well-stained section, should be placed on the stage and the objective focussed upon it. One with previous microscope experience will have an advantage here, but let us suppose that the tyro is just starting and knows nothing about the operation of the microscope.

With the object in place on the stage and the substage condenser racked up until it nearly touches the slide, the substage diaphragm being opened wide, the object will be brightly illuminated when the lamp is turned on. Care must be exercised at this point not to look into the eyepiece until means have been taken to reduce the intensity of the light to a point where it is safe for visual work. Possibly the light can be cut down by means of a rheostat provided for the purpose. If not, color filters or neutral-tint glass can be inserted in the light train, or extremely intense light can be reduced by the use of a dark glass eyepiece cap. When the light is properly adjusted, the eye can be placed over the eyepiece and the objective focussed on the object until the latter is sharp. With a 10x objective, this will be when the distance between the object and bottom of the lens is around $\frac{1}{4}$ " to $\frac{3}{8}$ ".

For the sake of emphasis we can ask a question here. Are we now ready to move the camera into place, turn the light on to full

brilliancy, focus the image on the ground glass, and expose for a picture? Many who have been engaged in taking photomicrographs for a long time might answer "Yes," or counter with a "Why not?" But the answer is decidedly "No." We have not yet assured ourselves that the light is critical. If the so-called Köhler system of illumination is to be used, as is the present common practice with many commercial outfits, the first step is to move the lamp condenser back and forth until the position is found where the light source, whatever its nature, is sharply imaged on the iris diaphragm of the substage condenser. The latter can be partially closed to accomplish the focussing, if necessary, or a piece of white paper held against it, more readily to discern the image.

When the lamp condenser has been designed for Köhler illumination, an iris diaphragm is usually mounted in front of it. This is the *field diaphragm*. It should be partially closed at this stage, in preparation for the next step, the focussing of the substage condenser. To accomplish this, look into the microscope and, after first making sure that the object is in accurate focus, move the substage condenser until the circle of light, limited in size by the partially closed field diaphragm, is also in sharp focus, as well as the object. The circle of light should appear evenly illuminated, although it may not be found to occupy the center of the field, nor to cover the entire field, which is more or less in darkness around it. Opening and closing the field diaphragm will increase or diminish the area of the field illuminated, thus demonstrating the propriety of its name. It is provided *for this one purpose*, that the actual part of the object illuminated need not be appreciably more than the size of the circle to be photographed. Any light not actually needed to take the picture should be eliminated, since it serves only to fog the image and lower the quality of the picture.

If the microscope is all that it should be for photomicrography, some means will be available for centering the objective to the condenser. The centering may be accomplished either by means of centering screws on the condenser, or by a centering device associated with the objective, such as an objective changer.* Ordinary nose-

* The specific type of centering device employed is frequently controlled by the type of microscope stand — i.e., whether it is one equipped with a square or fixed round stage, or a rotating stage. Where the stage is fixed, either the objective or the condenser may be provided with centering means, but rotating stages, unless equipped with their own centering screws, must be associated with a centering device on the body tube, so as to bring the center of rotation of the stage to the center of the ob-

pieces are not centering and hence are not suitable for photomicrography unless attached to the body tube by a centering device of some sort.

Assuming some centering means to be present, if the illuminated circle does not lie in the center of the microscope field (which indicates that the substage condenser and objective are not centered with respect to each other), it should now be brought there by manipulation of the centering screws. That lack of centrality does result from nonalignment of the objective and substage condenser can be demonstrated at this point, if desired, after the centering has been accomplished with one objective, by the simple expedient of substituting another objective for it. Only by rare chance will the second objective be found to have the illuminated circle lying in the exact center of its field.

One other adjustment must be made before the light is perfect. The angular aperture of the cone of light from the substage condenser to the object must be made to agree with that of the objective. To do this, remove the eyepiece and look down the microscope tube at the back lens of the objective. Now open and close the substage diaphragm. The movement of the diaphragm can be clearly seen, as the circle of illumination increases and diminishes accordingly. The position of the diaphragm should be set so that its edge can be observed as just coinciding in diameter with the back lens of the objective. The light is now "critical"; we have *critical illumination*. Because the substage diaphragm serves to vary the aperture of the system under this setup, it is called the *aperture diaphragm*.

Replacing the eyepiece will reveal the best possible resolution of the object, provided that the latter is a suitably stained section and not a slide of diatoms or other unstained object with little refractive index differentiation. The latter are not fitted for making a test of this sort, for reasons which will become apparent as we go on.*

One single condition in the illumination may be disconcerting to the photomicrographic novice. The entire field of view is probably not entirely covered, even with the field diaphragm opened to its fullest extent. This is not necessarily objectionable in photomicro-

jective field. In other words, there are three parts to be centered with respect to each other. To accomplish this result, two centering devices must be present. These can be: centering condenser and centering objective; centering stage and centering objective; or centering condenser and centering stage. Sometimes the more elaborate stands are provided with all three adjustments.

* See page 189.

raphy, because usually the entire field, not being required in the picture, need not all be covered by the light. An understanding of the reason for the non-coverage will show the logical method of overcoming the trouble. Although moving the condenser farther away from the slide will be found to increase the area of illumination — and this method is often followed by those ignorant of the correct operation of the microscope — *it should never be resorted to*, as it immediately destroys the critical setup we have taken so much pains to produce.

The reason for the small illuminated field lies in the design of the substage condenser, which has been provided with a large aperture so as to function with high-power lenses. When these higher-power objectives are substituted for the 10 \times , the field will be found to be fully covered. To take care of low-power lenses such as the 10 \times and lower, manufacturers usually so design the condenser that its top lens can be removed, or a supplemental lens used below it. Unscrew the top lens and repeat the entire series of operations, from the point of focussing the substage condenser. The field diaphragm should be closed materially for this purpose. The substage condenser will be found to focus at a point much farther away. The aperture diaphragm opening, which probably was only a few millimeters in diameter with critical lighting when the top lens was in place, now will be found to require substantial increasing.* After the new adjustments are completed, opening the field diaphragm will be found to cover amply the field of the microscope.

* From a strictly theoretical standpoint, when the top lens of the condenser is removed, accurate conditions for obtaining critical light by the so-called Köhler method no longer exist, since the substage condenser diaphragm ceases to lie in the rear focal plane of the condenser. It should be moved farther away to agree with the resulting longer focus, but microscopes are not provided with means for doing this. The effect of this condition is that the substage diaphragm is no longer a true aperture diaphragm and a slight vignetting effect is introduced. However, since we are dealing with objectives of relatively low aperture whenever the necessity of removing the top lens is present, the condition is not serious. It can be overcome to some extent, if one desires to approximate the ideal condition, by no longer focussing the image of the light source on the substage diaphragm, but on a card held some distance away, at a point substantially the same distance from the back of the condenser lens (consequently nearer the light source) as the object is from the front surface, when the condenser is focussed on the object. This causes the rays to cross more nearly at the point where the diaphragm should be located.

When the author's method of obtaining critical light, as described on page 218, is used, this complication is not a factor, since parallel rays are entering the condenser and the aperture diaphragm need not be located at the rear focal plane to secure the desired limitation in the cone of light.

What has been done is to substitute a longer-focus condenser, which yields a larger image of the light source (the lamp condenser, in this case). This same principle will be found to operate with still lower objectives, under this same setup, although a point is finally reached where a change to low-power Köhler illumination must be made if it is desired to utilize the entire field-covering capacity of the lenses. The practical application of this system will be taken up later; also, further information will be given on the alternate method of obtaining critical illumination by imagining the light source.* The relative advantages and disadvantages of the two systems will also be discussed. For the present, however, let us consider that we have laid the foundation for taking photomicrographs. Once we have arrived at this stage, the camera can be put into position, the strong light made available, and the image examined on the ground glass. When the image is in focus visually, it will *not* be in focus on the glass focussing screen, but a slight adjustment of the fine-motion screw will suffice to make the change from one to the other.

The effect of varying the bellows length on the size of the image should be noted, and also the variation in image size with various eyepieces. It will be found that considerable change can be made in bellows length without apparent effect on the sharpness of the focus. This may be a surprise to those experienced in ordinary photography. Different eyepieces, however, may require considerable alteration in the focus, but such alteration also can be effected by means of the fine adjustment of the microscope.

After becoming familiar with the operation of the outfit, using the low-power ($10\times$) objective, a higher power, say a $20\times$ or $40\times$, can be substituted.

Each step of the process of securing critical illumination should be repeated for every objective change, except that when the top lens of the substage condenser is in place and the same slide is used, it is necessary only to adjust the aperture of the condenser to that of the objective. Later on, for actual photographing, a final adjustment of the field diaphragm should be made in every case to assure that only the part of the field actually being photographed is illuminated.

No attempt should be made to take a picture until a number of other matters have been settled. Up to this point we can only say that the preliminary steps have been taken; we're off for a good start.

* See page 217.

Plates and Films for Photomicrography

Although the present tendency is toward the use of cut film as a substitute for the time-honored glass plate for most photographic work, microscopy is one exception; many photomicrographers still prefer plates. One reason for this is that certain brands of plates, of special value for photomicrographic work, are not furnished in sheet film. Then again, the advantages of sheet film for general work — lightness, where portability is a factor; small storage space required; possibility of bulk development in a tank, etc. — have no appeal to the photomicrographer. He does not need to carry them around to various outside jobs; he does all his work adjacent to the darkroom; he prefers to load a single plate at a time (in most cases), expose, and tray-develop it before taking the next picture; and ample storage space is usually available.

However, a new factor has entered the picture of recent years — an enormous increase in the cost of glass plates as compared with sheet film. This is forcing many who would prefer plates to use sheet film. The Eastman Company still provide plates for those who insist on using them at any cost, but usually they must be specially ordered, since dealers no longer stock them. Realizing the situation in which microscopists have been placed, the line of sheet film has been augmented to the extent that films with characteristics making them suitable substitutes for micrographic plates are now available.

No one type of plate or film will serve for all classes of photomicrographic work. On the other hand, the number of kinds which must be on hand to meet ordinary conditions should be kept at a minimum. Of course, those who specialize in but one line of work may often get along with one kind of plate or two, at the most. To cover the entire field (except for infra-red work), four different types will be found adequate. These, with their special characteristics, are as follows:

1) *The Kodak M plate (Eastman Kodak Co.).* This was developed especially for photomicrographic work. It is a panchromatic plate, covering all the visible spectrum, and slightly beyond, in both the ultra-violet and the infra-red. It is a relatively slow emulsion and possesses a fine grain; both of these characteristics are valuable in photomicrographic work. Moreover, it is fairly contrasty, which is

also often a desired feature in photomicrographic work. This plate should be kept on hand at all times, and if one were to standardize on but a single type of plate, in nine cases out of ten this one would give the most all-around service.

(2) *The Kodak Panchromatic plate (Eastman Kodak Co.).* This plate is much faster than the Kodak M plate, requiring only from 30 to 40 per cent the exposure time with tungsten lights, other conditions being equal. It is not, however, for its increased speed that this plate is used in place of the Kodak M. Its value in special cases lies in its greater softness, where the contrast of the M plate is excessive for proper rendition of all the tonal scale of the object. It is often desirable to sacrifice the fine grain of the M plate (for the Kodak Panchromatic is much coarser in this respect) in order to reduce the contrast unavoidably present in the object.

There is, of course, considerable latitude in the degree of contrast possible with either plate, through the use of the different developers recommended by the plate manufacturers; but in general it will be found that the extreme contrast procurable in the Kodak Panchromatic only slightly overlaps the ordinary soft development of the M plate. These two types of plates are priced the same, but are considerably more expensive than non-color-sensitized, or orthochromatic plates. This is one reason (but not the only one) why orthochromatic plates should be used when proper color rendition of the red is not required.

(3) *The Kodak Metallographic plate.* A third type of plate for photomicrographic use is supplied by Eastman. It is known as the Kodak Metallographic plate. As its name indicates, it is intended primarily for metallographic use. It is orthochromatic only and is quite contrasty, but of very fine grain — features desirable for this type of work.

(4) *The Process plate.* For some classes of work an extreme-contrast plate with no color correction is an important addition to the photomicrographer's stock. For these, Process plates, which are of very fine grain and extremely slow, are ideal.

These four types of plates are now practically duplicated in sheet film, and by reference to the table, Characteristics of Kodak Photographic Materials, films corresponding in characteristics to the four plate types listed can be selected. A good substitute for a large percentage of photomicrographic work is the Panatomic X Film,

CHARACTERISTICS OF KODAK PHOTOGRAPHIC MATERIALS^a

Material	Color Sensitivity	Relative Speed		Gamma Value	Contrast	Resolving Power lines per mm 30:1 test object	Safelight
		Minimum Brightness Criterion	Maximum Brightness Criterion				
Sheet Films							
Contrast Process Pan	Type B panchromatic	3	8 ^b	4.75	High	120	3
Contrast Process Ortho	Orthochromatic	2.5	6 ^b	4.25	High	125	1
Super Panchromatic	Type B panchromatic	100 ^b	125	0.9	Low	80	TD(3)
Super Ortho Press	Orthochromatic	50 ^b	60	—	Low	95	2
Commercial	Blue-sensitive	6 ^b	8	1.3	Medium	85	1
Tri-X Pan	Type C panchromatic	160 ^b	—	0.75	Low	65	TD(3)
Ortho-X	Orthochromatic	64 ^b	—	0.75	Low	85	2
Roll Films and 35-mm Films	Type B panchromatic	40	—	0.8	Low	95	TD(3)
Plus-X	Type B panchromatic	80	—	0.8	Low	90	TD(3)
Super-XX	Orthochromatic	32	—	0.8	Low	95	2
Verichrome (roll only)					—	—	—
Color Films					—	—	—
Ektachrome, Type B	—	—	10	—	—	100	3
Kodachrome, Type A	—	—	16	—	—	—	—
"M"	Type B panchromatic	8	80	2.0	High	—	—
Metallographic	Orthochromatic	8	160	3.0	—	115	2
Panchromatic	Type B panchromatic	6	60	2.0	High	—	—
Process	Orthochromatic	6	120	3.0	—	80	TD(3)
Super Ortho Press	Blue-sensitive	10 ^b	15	0.9	Low	—	—
Tri-X	Type B panchromatic	0.6	12 ^b	3.0	Medium	80	2
		50 ^b	8	1.2	Low	85	1
		5 ^b	—	0.9	—	—	TD(3)
		100 ^b	—	0.75	Low	75	

^a From Eastman Kodak Company, *Photography Through the Microscope*, by permission.^b These values correspond to the published Exposure Index.

which is not listed in this table. It is intermediate in speed, grain, and contrast between the M plates and the Panchromatic plates, and hence can usually replace both.

One advantage of sheet film over plates is that antihalation backing usually is unnecessary. It is the common practice at the present time to back all glass plates, as well as a few types of sheet film, where halation could result and absolute antihalation is desirable.

Since many recent models of photomicrographic cameras are equipped with backs taking roll film or film packs, users of these must be content with such films as are available. Where cameras are supplied with individual plate holders they are usually to be preferred because of the ease with which single negatives can be taken and developed. The table lists the various types of roll film available from Eastman. Other film manufacturers supply equivalents. Sheet film can be obtained in the form both of cut film and of film packs.

Special branches of photomicrography — i.e., those discussed in Chapter 5 — may require other types of plates, but these will be indicated in connection with the particular work for which they are needed.

Regardless of the brand or type of plate being used, it is important that each individual plate be brushed off with a fine camel's-hair brush immediately before being placed in the holder; otherwise dust particles may be present and cause pinholes. Most plates might not require brushing, but unfortunately one cannot tell in the darkroom whether or not it is needed, and so, to be on the safe side, always use the brush or some equivalent method to assure freedom from dust. Occasionally a particular box of plates will be found unusually dusty.

One reason for standardizing on a minimum in the varieties of plates used is the fact that plates do not keep indefinitely without some deterioration. Fresh plates are to be preferred for best performance. There is, however, considerable difference in the keeping properties of plates. In general, panchromatic plates do not keep as well as non-panchromatic, and fast plates deteriorate more quickly than slow. Keeping depends largely on the storage conditions. The ideal method of maintaining freshness is to store the reserve supply in a closed metal container in a refrigerator. Even infra-red plates keep well under such conditions.

Fortunately the photomicrographer is not ordinarily interested in supersensitive plates, which are the worst of all from a keeping standpoint.

One other thing which can be done to reduce further the stock kept on hand is to standardize on one size and consider all others as special. Where the camera will accommodate large plates, kits can be used

and the larger sizes reserved for unusual occasions. For commercial work the 5" x 7" size is ideal, but both 4" x 5" and 3 $\frac{1}{4}$ " x 4 $\frac{1}{4}$ " pictures are satisfactory for many purposes and are less expensive.

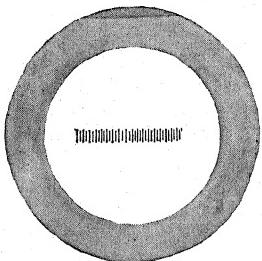


FIG. 81. Stage Micrometer, Enlarged about Ten Diameters

list is a stage micrometer, for the purpose of determining the magnification of the particular objective, eyepiece, and bellows-length combination being employed. This is a scale ruled in hundredths of a millimeter on a standard 3" x 1" glass slide. It is illustrated in Figure 81. An eyepiece micrometer is not an essential for strictly photographic work, but is required for visual determination of magnification. Sometimes it happens that a net ruling (graticule) or some other scale is required to be photographed superimposed on the object, in which case a standard micrometer eyepiece with adjustable eye-lens must be available.

The next items on the list are a focussing glass and an ordinary circular reading glass. For the former a 6x aplanat in a focussing mount is usually employed. The Bausch & Lomb model is shown in Figure 82. The focussing feature is essential when it is desired to examine the image through clear glass, as will be explained later. For some work, a three-inch reading glass serves better for checking the focus when the ground glass screen is used. It enables a general survey of the entire area to be made and yields a composite view much superior to that of the higher-power focuss-

Miscellaneous Equipment for the Photomicrographic Table

A few additional pieces of equipment not recognized as part of the photomicrographic apparatus are nevertheless almost indispensable to the photomicrographer. First on the



FIG. 82. Bausch & Lomb Focussing Glasses

sing glass, when a thick or wavy section is being photographed.

A Kodak Timer (Figure 83) or its equivalent is a desirable adjunct to the outfit. Usually one is dealing in seconds, or split seconds, in exposure, and hence a timing device where the seconds are easily read is superior to an ordinary watch.

A good stop watch is satisfactory but the larger clock dial can be seen at a much greater distance. Other useful articles include: a hand mirror for observing the image on the focusing glass screen at the same time adjustments are being made on the optical bench or where it is not possible to see the image; an ordinary millimeter ruler; a pair of machinist's dividers for measuring the image on the ground glass when the light in the room is not sufficient to use the ruler directly; an inexpensive slide rule, for computing exposures; and printed record cards for keeping permanent data on every exposure made.

A copy of the record card used by the author is shown in Figure 85. Other workers might desire to keep data not provided for on this card; each should therefore design a card to suit his individual needs. They can be printed to order by any job printer; there are none available in the market.

A combination immersion oil and xylol bottle such as that supplied by Zeiss (Figure 84) should be at hand, for there is a constant demand for xylol, as well as for the oil, especially when immersion work is being done. Separate bottles can, of course, be used, but as the Zeiss design requires so little room and the base is so broad it is almost impossible to upset it, it is a particularly good one.

Finally, a pair of soft camel's-hair (or sable) brushes should be kept on the photomicrographic

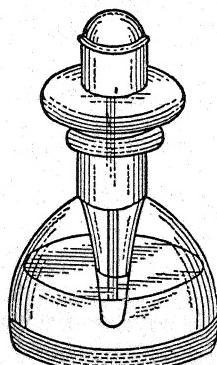


FIG. 84. Zeiss Bottle
for Immersion Oil



FIG. 83. Kodak Timer

table, for dusting purposes. One should be a flat brush, about $\frac{3}{4}$ " to 1" wide, for dusting the larger condensing lenses, which tend to draw dust, lint, etc., because of electrostatic attraction. The other brush should be a small, round, long-handled artist's pencil brush, for re-

PHOTOMICROGRAPH RECORD		
NEGATIVE NO.....	DATE.....	
SUBJECT.....	TRANSPARENT.....	
.....	OPAQUE.....
MAGNIFICATION.....	DIAMETERS.....	DARK FIELD.....
OBJECTIVE.....	EYEPIECE.....	CAMERA HOR.....
CONDENSER.....	ILLUMINATION.....	VERT.....
PLATE.....	FILTER.....	EXPOSURE.....
REMARKS.....		

FIG. 85. Photomicrograph Record Card

moving lint from the eyepiece, object cover glass, or, when necessary, the back lens of the objective. It is usually necessary to brush the lint from the eye lens of the eyepiece each time it is used, unless the room is free from dust.

These few items will be found sufficient for general work, although it is only to be expected that many others will be required occasionally, for specific purposes.

Photomicrographic Optical Equipment

The usual microscope employed for visual work is equipped with three objectives, a 16-mm. (10 \times), a 4-mm. (40 \times), and an oil-immersion 2-mm. (90 \times). These, supplemented by two, or at the most three, eyepieces — 5 \times , 10 \times , and either a 7.5 \times or a 15 \times — constitute the total opti-

cal equipment in the vast majority of cases. It is a safe estimate that hundreds of microscopists, considering themselves in the photomicroscopic class because they are provided with an outfit for taking pictures, have no other optical facilities than the three-objective, two-eyepiece combination.

Such a combination is extremely limited, when the entire gamut of magnification range is considered. Even if the amount which can be invested in photomicrographic equipment be limited, if at all possible it should be supplemented by a $20\times$ (8-mm.) objective and one of very low power, say a $3\times$ or $5\times$. As a matter of fact, it would make a better combination to replace the $40\times$ objective by the $20\times$ and $5\times$, for the latter will prove more useful in the long run.

Where one may have a fairly complete line of optical equipment, the entire range can be covered effectively with the lenses listed in the following table.

LIST OF USEFUL LENS COMBINATIONS

Objective	N.A.	Eyepiece	Approximate Range, Diameters
6" Goertz Dagor	.07	—	1-4
100-mm. Planar	.11	—	2-9
75-mm. "	.11	—	4-13
50-mm. "	.11	—	5-22
35-mm. "	.11	—	10-30
20-mm. "	.11	—	20-57
16-mm. Spencer Photo ^a	.25	—	25-75
6x Apochromat	.15	Homal II	30-100
6x "	.15	" I	80-300
10x "	.30	" II	55-160
10x "	.30	" I	125-475
20x "	.65	" II	105-350
20x "	.65	" III	250-1000
40x B & L Fluorite Immer. ^b	1.00	" IV	500-2000
60x Apochromat Immer.	1.05	" IV	800-2800
90x " "	1.40	" IV	1100-4500
120x " "	1.30	" IV	1500-6000

^aThis is an old-style photographic 16-mm. objective, made by the Spencer Lens Co. It is equipped with an iris diaphragm and designed for use without an ocular. Except for considerable curvature of the field, it is a beautiful lens, within its range.

^bThis lens of the Bausch & Lomb line, although rated only as a fluorite, yields a magnificent image, far superior to any dry 4-mm. apochromat made.

This list represents those from the author's own extensive series with which at least 95 per cent of his photomicrographs are taken.

The approximate range given in the last column is based upon a maximum camera extension of 112 centimeters (44 inches) and a minimum picture size of $3\frac{1}{2}$ inches in diameter. With a lesser bellows extension the maximum magnification is reduced in proportion, but should a smaller picture than $3\frac{1}{2}$ inches in diameter be satisfactory, the low-range figures can be extended farther downward.

It is evident that with such series of lenses available, in combination with a long bellows extension, considerable overlapping in magnification is provided. This is a valuable asset for meeting numerous conditions that arise. The theoretical reason for this was outlined in Chapter 1; we can now see how it works out in a practical way. To do this it is desirable to formulate a few rules, which are worth remembering. First, some definitions may be in order.

Magnification, it will be evident, is the number of times larger (in lineal dimension) the image (i.e., the photomicrograph) is over the object. That is,

$$\frac{\text{image size}}{\text{object size}} = \text{magnification.}$$

The *field diameter* is the actual diameter of the illuminated circle seen when looking into a microscope, the *field* itself designating whatever is included within the circle. If the object were circular and just the exact size to fill the field, *field diameter* and *object size* would be identical, and the terms could be used interchangeably.

The *projected image diameter* is the diameter of the circular image of the field as it is projected on the focussing screen of the camera.

The *camera extension* is the distance from the eyepiece (or, to be more specific, the eye-point, just in front of the eyepiece) to the focusing screen (i.e., the position of the plate).

Then our first axiomatic rule is: *With a fixed projected image diameter, the field diameter is the same for a given magnification, regardless of the objective, eyepiece, and camera extension being employed.* This follows from the definition of magnification.

One condition confronting the photomicrographer, in every picture taken, is the order of magnitude of the object to be shown. It may be a large object, several millimeters in diameter, or only a minute fraction of a millimeter. The diameter of the field he must include is therefore the very first matter to settle. Each objective will cover

only a small portion of the total range from largest to smallest object.

A corollary of the first rule can be stated thus: *With a constant projected image diameter, as the magnification is increased, the field diameter decreases. Conversely, as the field diameter is increased, the magnification decreases.*

Working these rules out in their application to the series of objectives previously tabulated, assuming a fixed projected image diameter of 5 inches (i.e., a size to cover a 4" x 5" plate or a 5" circle on a 5" x 7" plate), the field diameter, in millimeters, at various magnifications, is as follows:

FIELD DIAMETER (IN MILLIMETERS) AT VARIOUS MAGNIFICATIONS

EQUIVALENT FOCUS OF OBJECTIVE	MAGNIFICATION													
	1	5	10	25	50	100	200	500	1000	2000	2500	3000	4500	6000
6 inch	125	25												
100 mm.		25	12.5											
50 mm.		25	12.5	5	2.5									
20 mm.				5	2.5	1.25	.62							
25 mm. (6x)						1.25	.62							
16 mm. (10x)						1.25	.62							
8 mm. (20x)						1.25	.62	.25	.125					
4 mm. (40x)								.25	.125	.062				
3 mm. (60x)									.125	.062	.05			
2 mm. (90x)										.125	.062	.05	.042	.028
1.5 mm. (120x)											.062	.05	.042	.028
Number of fields in a lineal inch		1	2	5	10	20	40	100	200	400	500	600	800	1200

In this table we see exemplified the two rules enumerated, as well as the fact that the same magnification, with its corresponding field diameter, can be obtained by several overlapping objectives. There is here, however, no mention of how the ultimate magnification is obtained — that is, what part the eyepiece and camera extension contribute toward it. The last line, giving the number of field diameters occurring in a single inch, will aid in visualizing the actual size of the field. When it is realized that at a magnification of 6000 \times the diameter of the entire field shown in a five-inch circle is so minute that 1200 of them could be placed in a row in the space of one inch, one obtains an idea of the extreme minuteness of the area portrayed. Should it be

desired to photograph every part of an object one inch long, 1200 five-inch pictures must be taken, which, if placed end to end, would extend five hundred feet!

In leading up to a consideration of the value of overlapping objec-

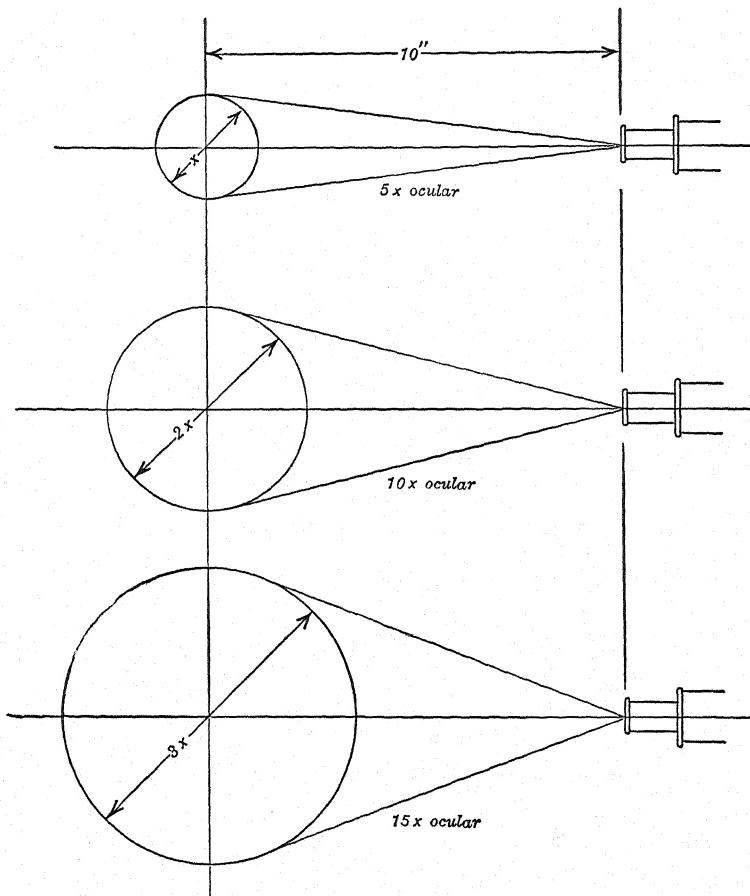


FIG. 86. Effect of Eyepiece on Magnification

tives in photomicrography, as the combination of eyepieces and camera lengths play an important part in the result, we must examine the effect obtained when different-power eyepieces are used.

The magnification of the projected image at a distance of 10 inches from the eye-point is identical with that of the visual image. Conse-

quently at a 10-inch camera extension the image produced by a $10\times$ ocular will be double that of a $5\times$ ocular with the same objective. This can be brought about in only one way: the angle of the higher-power eyepiece must be greater than that of the lower. In other words, the diameter of the projected image is increased. Figure 86 illustrates this condition. At A, B, and C are shown the spread of the image-forming rays of $5\times$, $10\times$, and $15\times$ oculars and the resultant size of the projected image at the 10-inch distance.

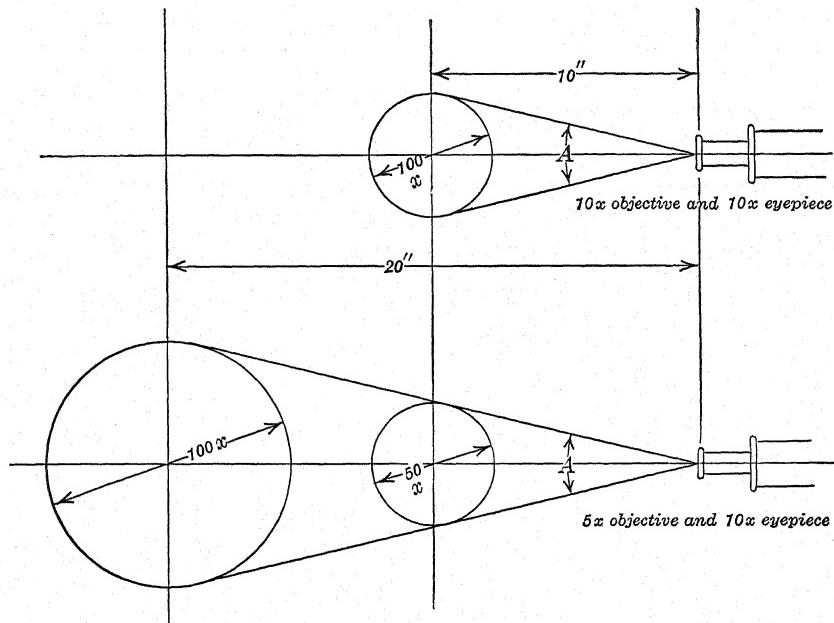


FIG. 87. How a Lower-Power Objective Covers a Greater Plate Area for a Given Eyepiece and Magnification

From this diagram it might be assumed that measurement of the projected circle on the ground glass would show the same relative proportion in size, but this is not the case. The diagram is predicated upon the field diameter's being allowed to remain the same. Actually the field diameter is circumscribed by the diaphragm in the eyepiece. This diaphragm restricts the diameter of the field to a greater extent in the higher-power eyepieces, and hence there is not a proportionate increase in the size of the circle. Measurement of the diameter of any object lying within the circle, with various power oculars, will demon-

strate that the angle does increase proportionately with the magnifying power of the ocular.

When we consider superimposing on this eyepiece effect a variation in the camera length, the result indicates the flexibility possible in obtaining a desired effect.

Figure 87 shows the effect upon the image of the same eyepiece when used with different objectives and varying the camera length to obtain the same magnification. The striking difference between the two conditions is the increased size of the projected image diameter for the same magnification, with the lower-power objective.

Thus we see that even when similar magnifications are producible by several different objectives, it does not mean duplication of equipment, for there is always some variable in the final result that is of value under circumstances continually arising in photomicrography. The extent of variation in the size of the final picture required, with other characteristics constant, ranges from the covering of an 8" x 10" plate, to a single frame of 16-mm. motion-picture film. Only with a complete set of objectives and eyepieces can such wide range be accommodated.

The table opposite will be of value in supplementing those already given. It gives both the magnification (in parentheses) and the field diameter in millimeters of the commonly used objectives and eyepieces at a projected image distance of 10 inches. The field diameter is computed on a 5-inch diameter for the image, although the 5x ocular will not usually yield a circle larger than about $3\frac{1}{2}$ inches at the 10-inch distance. For other sizes of image circles (X inches in diameter) at the same projection distance, to find the actual field included,

multiply the given field diameter by $\frac{X}{5}$. Should the same field diameter be desired, but a different size image required, the magnification will be the factor affected through change in the projection distance. To find the magnification in this case, multiply the figure given by projection distance.

10

From what is given in the table we can add a few simple rules:

With a given objective and eyepiece, magnification varies directly as the projection distance from the eye-point (Ramsden circle).

Based upon this fact, a simple method of graphing magnification is possible. This method will be described a little later.

With a given eyepiece and magnification, the actual field diameter is inversely proportional to the initial magnification of the objective.

With a given objective and fixed projection distance, the magnification and projected image diameter (usually) are increased by the use of higher-power eyepieces.

MAGNIFICATION AND FIELD DIAMETER (IN MILLIMETERS)
FOR A PROJECTED IMAGE AT 10 IN.

OBJEC- TIVE	OCULAR					
	5x	7.5x	10x	12.5x	15x	20x
5x (25x)	5 mm. (37x)	3.33 1.67	2.5 (100)	2.0 (125)	1.67 1.0	1.25 0.83
10x (50)	2.5 (75)	1.67 1.0	1.25 (200)	1.0 0.625	0.83 0.50	0.62 0.42
20x (100)	1.25 (150)	0.83 0.53	0.625 (250)	0.50 0.35	0.42 0.25	0.31 0.21
40x (200)	0.625 (300)	0.42 0.28	0.31 (400)	0.25 0.21	0.21 0.167	0.16 0.14
60x (300)	0.42 (450)	0.28 0.185	0.21 (600)	0.167 0.14	0.14 0.111	0.10 0.07
90x (450)	0.28 (675)	0.185 0.14	0.14 (1200)	0.111 0.10	0.11 0.083	0.07 0.05
120x (600)	0.21 (900)	0.14 0.14	0.10 (1500)	0.083 0.07	0.07 0.05	0.05

Regardless of the type of camera employed — whether minicam, fixed projection distance camera, motion-picture camera, or large research model — application of these rules will enable one to determine how to obtain the maximum variation with the equipment available.

When special flat yield eyepieces of the Homal type (see pages 89 and 185) are employed instead of the ordinary series, the flexibility obtainable by substituting different eyepieces is materially limited, so that these are not so ideal for very simple cameras. Their use is largely confined to the elaborate research models.

Also, there is not the flexibility of the compound microscope where low-power lenses are required, as these use no eyepiece. Hence the steps between the focal lengths supplied are graded much closer than regular objectives. Starting with a 32-mm. (5x) in the latter, the steps are in the ratio 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, while a series of five Planars, starting with 100 mm., is available in the ratio: 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{3}$, $\frac{1}{5}$. In this way suitable flexibility is provided.

Magnification—Its Practical Determination

It is important to know and record the magnification employed for every photomicrograph taken. In setting up for the picture, two con-

ditions are possible. The first is that the object, with a suitable margin around it, is required just to fill an image circle of a predetermined diameter. The diameter is usually set by the size of plate or film determined upon. The exact magnification necessary to accomplish this is inconsequential, except that after the picture is taken, it is then desirable to know the magnification actually used, both for the purpose of recording on the back of the finished prints and for permanent record.

The alternate condition is to predetermine the magnification most satisfactorily showing the subject, and then establish the combination of objective, eyepiece, and bellows length giving the desired result. In general this latter method is used for subjects not possessing a definite outline or circumscribed area — extended surfaces, tissues, smears, particles, etc. — and the magnifications employed are round numbers. In the former case the objects are entities which must be shown entire, yet at the highest possible magnification for the sake of maximum detail.

Means must therefore be available in both instances to determine the magnification; it is not sufficient to depend upon computations based upon the magnification engraved on the objective and eyepiece, and the measured bellows extension. This would give an approximation only.

For the measurement of magnification, a stage micrometer is a necessity. This is in effect a minute ruler, on glass. The scale is usually 2 millimeters long and subdivided into .01 mm. By placing the stage micrometer (Figure 81) as an object on the microscope stage and projecting its image on the ground glass focussing screen, the amount each division of the scale is enlarged represents the magnification. Two other inexpensive pieces of equipment are required. One is a ruler, graduated in millimeters, and the other a simple pair of dividers (a draftsman's compass with points, or machinist's dividers). The latter is useful in spanning the scale divisions as seen on the ground glass and transferring them to the ruler, in lieu of superimposing the ruler directly on the image of the scale.

Instead of measuring the image of a single division, it is better to include as many as possible, to minimize error in the reading. For instance, if 10 divisions on the image (each representing $1/100$ of a millimeter) measure 2 centimeters (20 mm.) across, one division would equal $1/10$ of 2 cm., or 2 millimeters. To enlarge $1/100$ of a millimeter

until it equals 2 mm. requires a magnification of $\frac{2}{1/100} = 200x$.

Many workers place the scale in position each time a picture is taken, to obtain the magnification. This requires considerable time and is unnecessary. It is a simple matter to determine the magnification for each combination of objective and eyepiece once for all, and then plot the results on a piece of graph paper for ready reference. Figure 88 shows such a graph as it appears when ready for use.

According to the rule already stated — that with any given objective and eyepiece, the magnification varies directly as the distance of the projected image from the eyepoint — the magnification relationship is a straight line. One need only establish two points on the graph and connect them by a straight line to indicate the magnification at any projection distance. Though this also holds good for extrapolation on both sides of the points determined, it is preferable to establish the two points at as near the limiting positions as possible.

The camera extension positions, indicated at the bottom of the graph, will be identical in every graph, for the same camera, regardless of the lens combination, but the spacing of the magnification values will require recomputing for each setup. The subdivisions for the magnification should be such that intermediate values are easily determined — that is, they should represent steps easily divisible into the base figures, 1, 10, 100, or 1000, as the case may be. In the graph shown each group of five lines represent 50x of magnification; the lines, therefore, are in steps of 10x.

The shortest projection distance possible, with bellows-type cam-

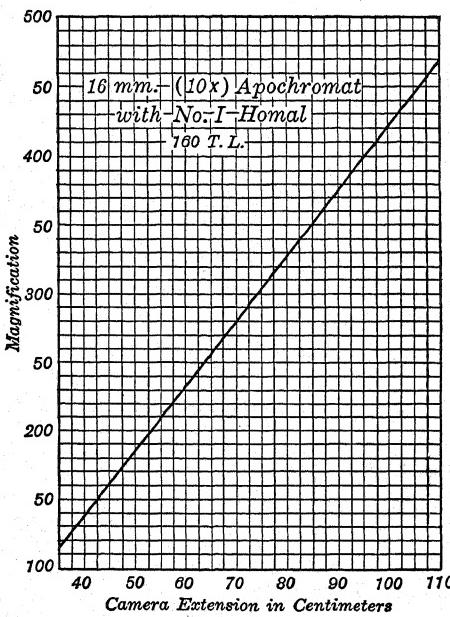


FIG. 88. Typical Graph of Magnifications with Given Objective and Ocular

eras, is the distance to the plate when the bellows is completely collapsed, and so the graphing of the magnification never need go below this point.

Workers using fixed-focus cameras need not prepare separate graphs of magnifications, for a single table can show every possible combination. Reliance should not be placed on manufacturers' figures, which are approximate only; even with roll-film minicams the exact magnification should be determined for every combination. This can be accomplished by removing the camera back and laying a piece of fine ground glass, ground side down, over the film guides. The stage micrometer can be projected onto this and measurements made in the way described for larger cameras.

Filters and Their Characteristics

Competence in photomicrography requires some knowledge of the nature of light, especially visible light. Light is the medium employed for producing the image on the sensitized plate or film, and therefore one must know how to control it. That portion of light which can be picked up by the human eye is composed of vibratory energy possessing wave lengths between about 400 to 700 $\text{m}\mu$ in length. These wave lengths of light energy, if allowed to fall on the eye in very narrow bands (or groups) produce the sensation known as color. The shortest waves are violet, the longest red. Between these extremes lie all the colors constituting the spectrum (e.g., the rainbow).

Only when all the rays composing the visible spectrum are present is the sensation experienced that is known as white light. Taking out any rays gives some kind of a color effect to the remaining light; the exact color depends, of course, on which wave lengths have been eliminated.

Transparent substances are those which allow light to pass through them. When every wave length can pass through with equal ease, the light transmitted appears the same as the light entering the substance. We then say the substance is colorless. Many transparent substances, however, will allow only certain wave lengths to pass through; the others, either wholly or in part, are absorbed by the substance and so never get through to the other side. The light passing through is said to be *transmitted*; that not transmitted is *absorbed*.

When a piece of glass appears red, it is because this is the only color transmitted; all the rest of the rays constituting white light are absorbed. Similarly, a blue glass transmits only the blue rays. Hence if we pass white light first through a red glass and then attempt to pass the transmitted red rays through a blue glass we find that the blue glass absorbs all the red rays and no light whatever gets through. On the other hand, replacing the blue glass with a second red glass allows the red rays to pass through unhindered. This transmission and absorption principle is employed extensively in photomicrography to obtain the desired image of a colored object.

Since a large proportion of objects which must be studied microscopically are colorless, they require artificial coloring (staining) to make them visible. Even blood, which we are accustomed to consider a rich red, when spread out in a single layer of corpuscles, on a slide, is nearly colorless and must be stained before it can be properly studied. Staining technique has been developed to such a high state of perfection, through the reaction of dyes with various tissues, that several different colors are possible on one section of tissue.

When these are to be photographed and interpreted in black and white, the various colors present require rendering in some intermediate half-tone which conveys to the mind an impression similar to the colors in the object itself. For this reason color filters find extensive use in photomicrography.

One factor in both naturally and artificially colored objects, as well as in color filters, of great value in securing the desired results, is that the line between transmission and absorption is seldom sharp. From substantially perfect transmission there is often a gradual increase in absorption until the maximum is reached. Moreover, this maximum is seldom 100 per cent absorption; it may vary anywhere between a low value and 100 per cent. These conditions will be evident from a study of the characteristics of color filters as illustrated by graphic means.

The filters employed in photomicrography are of three kinds: liquid, dyed gelatin, and glass. All three function in the same way; the advantages and disadvantages of each are largely the determining factors in the extent of their use. Liquid filters are solutions of colored chemical salts in water. The solutions are inexpensive but require a suitable glass cell for containing them. Such cells are expensive and if a complete set of solutions is kept on hand for immediate change, a cell is needed for each color. This is as costly as the purchase of gelatin or

glass filters. Some workers employ the cooling cell as the container for the filter solutions, but this involves a lot of work in changing from one color to another.

One advantage of solutions is the ease of altering the concentration of the chemical compound and thus securing an immense number of variations in color. The absorption of the solution changes with the concentration. A serious objection to the use of liquid filters is the difficulty of maintaining uniform standard solutions. Evaporation alters the concentration and sometimes chemical changes occur which affect the color. Unless a color comparator is available to keep check on the densities, exposure times will be affected.

Dyed gelatin filters are produced in the form of thin sheets, made by the Eastman Kodak Co., under the name Kodak Wratten filters. They are procurable either unmounted, in the thin sheet form, or mounted in an improved synthetic cement, between pieces of optical glass. The latter are preferable, as the unmounted sheets, though much cheaper, must be protected against scratches and finger marks by glass plates.

A set of nine filters, in a compact box was furnished for some years especially for microscopical work. It was known as the M set. The code letters and colors in this set were as follows:

1	A Filter	Bright red
2	B "	Green
3	C "	Blue-violet
4	D "	Purple
5	E "	Light orange red
6	F "	Dark red
7	G "	Orange
8	H "	Deep blue
9	K-2 "	Pale yellow

This set, boxed as a unit, is no longer available as such, but the same filters (with minor changes) are still recommended for photomicrographic work. Instead of being designated numerically from 1 to 9, in letter code A to H, and K-2, they are now known by their Filter Number as shown in the first of the accompanying tables. In addition to these some of the other Wratten Visual M filters, as listed in the second table, are suitable for photomicrographic work also.

While there is considerable variation in the filter factors when used

TRANSMISSION CHARACTERISTICS OF WRATTEN "M" FILTERS*

Kodak Wratten Filter Number	Visual Color	Spectral Transmission
25	Orange-red	From 590 m μ into the infra-red
58	Green	From 480 m μ to 620 m μ
47	Blue-violet	From 370 m μ to 510 m μ
35	Purple	From 320 m μ to 470 m μ and from 650 m μ into the infra-red
22	Orange	From 550 m μ into the infra-red
29	Pure red	From 610 m μ into the infra-red
15	Strong yellow	From 510 m μ into the infra-red
45	Blue	From 430 m μ to 540 m μ
11	Pale green	For correct tone reproduction with tungsten light

* From Eastman Kodak Company, *Kodak Wratten Filters*.

with different plates and films, the approximate values as given in the table, page 154, Filter Factors, are valuable for computing exposures. (Microscopists should secure a copy of the latest edition of Eastman's publication *Kodak Wratten Filters for Scientific and Technical Use*, available in all camera stores.) The time factors on these filters, for tungsten and arc lamps and for different types of plates, as furnished by the Eastman Kodak Co., are given in this table.

Glass filters, in anything like a complete series, are made only by Schott & Gen, the Zeiss optical glass plant, at Jena.* They have optically ground surfaces and can be obtained with practically any desired characteristics. It is possible to obtain an equivalent set in glass, to correspond with the Eastman Wratten M set.

As to the relative advantages of the dyed gelatin and glass filters, both have certain things in their favor and also some objectionable characteristics. The glass filters are much more expensive and more easily broken, especially the very thin ones (1 mm.). On the other hand, they are absolutely permanent, while some of the dyed gelatin filters will deteriorate rapidly under intense light. Excessive heat is hard on the cemented gelatin filters, causing the filter film to soften and run, but if heat of the same intensity be applied unevenly to the glass filters (as is nearly always the case with a focussed beam), the danger of breakage is great. On the whole, the final determining

* The Corning Glass Works also make many special glass filters.

TRANSMISSION CHARACTERISTICS OF WRATTEN
VISUAL "M" FILTERS*

Kodak Wratten Filter Number	Color	Use
78AA	Blue	A photometric filter which can be used to convert the color quality of light from incandescent tungsten lamps of the common type to that which is approximately visually equivalent to daylight. Often employed for viewing colored specimens with their commonly accepted standard daylight appearance.
38A	Blue	A filter for increasing the apparent contrast in faintly stained yellow or orange preparations. Helps in the resolution of fine detail.
45A	Blue-green	Especially useful when the highest resolving power visually possible is required, as in the study of diatom structure. It has no red transmission and its dominant wavelength range is 470-480 m μ .
66	Light green	A contrast filter for use with pink- and red-stained preparations. Preferred by some workers for general use in place of No. 78AA.
58	Green	A contrast filter for use with faintly stained pink or red preparations.
15 22	Yellow Orange}	For increasing the contrast in blue preparations and for helping in the observation of detail in insect mounts by reducing the contrast between the preparation and the background.
25	Red	Contrast filter for use with preparations stained with Methylene Blue, Methyl Green, etc.
96	Neutral	A filter for reducing the intensity of the illumination. The density supplied (1.0) transmits 10 per cent of the incident light.

* From Eastman Kodak Company, *Kodak Wratten Filters*.

factor in deciding which type to purchase will probably be the relative cost.

As to the exact correspondence of the glass filters to the Wratten M set, it must be recognized that the eye, unless specially trained, is unable to appreciate subtle differences by visual examination alone. When there is an appreciable difference in shade of color, the eye can detect it, but not the degree to which the transmission characteristics of filters may vary.

To determine this accurately, recording spectrophotometers are available, which automatically plot the transmission curve of any given filter. As there is very little comparative data available, covering the various filters used for microscopical work, the author, with the collaboration of the Harmon Color Works,* has worked out a representative selection of filters — liquid, gelatin, and glass — upon which graphs have been made on a General Electric Recording Photometer. These graphs are shown in Figures 89 to 103. To accompany these, corresponding curves were run on several of the common stains employed in histological work. These are shown in Figures 104 to 111. Though the curves of the stains represent definite concentrations of the dye solutions, these cannot be taken as the absolute equivalent of sections stained with these dyes. In many cases the dyes react with the tissues to produce a modified color. This is especially true in the case of hematoxylin, where the reaction results in a visual variation extending all the way from a reddish purple to a rich blue, although no blue exists in the stain. In spite of these variations, the curves of the dye solutions should prove helpful in determining which filter will be most likely to give a desired result.

The general law to follow in the selection of a filter is that one with a transmission curve similar to that of the color of the object will give the greatest amount of light through the object, with, consequently, the least amount of contrast and the maximum detail. Such a filter is known as a supplementary filter.

For maximum contrast a filter which absorbs the light the object transmits will give the best results. A filter of this type is called a complementary filter. Where an object has been double stained — e.g., blue and red, as in the case of hematoxylin and eosin — a filter intermediate between the two colors will give the best results. This

* A subsidiary of the Goodrich Tire & Rubber Co.: *The Goodrich Chemical Co., Harmon Colors.*

THE TECHNIQUE OF PHOTOMICROGRAPHY

FILTER FACTORS FOR PHOTOGRAPHIC EMULSION AND FILTER COMBINATIONS
MOST FREQUENTLY USED IN PHOTOMICROGRAPHY^a

FILTER	ORTHO		PAN B		PAN C	
	Tungsten ^b	Arc ^c	Tungsten ^b	"M" Plate Panchromatic Plate Tri-X Panchromatic Plate Contrast Process Pan Film Panatomic-X Film Super Pan Press Film	Tungsten ^b	Tungsten ^b
25	—	—	4	5	3	4
58	5	8	6	8	8	9
47	5	6	10	10	10	9
49	12	16	25	20	24	20
22	—	—	3	4	2	3
29	—	—	8	10	6	8
15	4	6	2	3	2	3
45	8	12	16	16	14	17
11	3	4	3	4	4	5
13	4	5	4	5	5	6
6	2	3	1.5	2	1.5	2
8	3	4	1.5	2	1.5	2.5
25-35	—	—	60	75	40	50
58-22	—	—	80	100	75	90
58-15	8	12	10	12	9	12
58-45	70	110	90	110	100	120
47-45	20	25	40	32	35	30
49-45	60	75	120	100	120	100
35-45	400	500	600	800	600	800
15-45	220	350	300	350	350	400
40	3	5	4	5	5	6
61	6	10	8	10	10	12
74	32	50	50	60	60	70

^a From Eastman Kodak Company, *Photography Through the Microscope*.

^b Tungsten source (3200K).

^c Cored carbon arc source.

should be a green filter for maximum contrast, or an orange filter for diminished contrast.

Filters are useful, not only in connection with stained objects, but for providing the sharpest image with achromatic objectives when photographing such subjects as diatoms, for maximum resolution. Usually, for this class of work, a green screen gives superior results. This is because the spherical and chromatic aberrations are corrected to the highest degree in the green region, so that if all light other than green be cut out, an achromatic objective will yield a picture almost the equal of an apochromat, under the same condition of illumination. The superiority of the apochromat is demonstrated in its ability to render just as good results in the blue-violet region, where the resolution is higher because of the shorter wave length.

Filters can also be used to reduce the intensity of a strong light when making a preliminary visual study of an object preparatory to projecting the image on the ground glass. The special filter, the curve of which is given in Figure 103, is particularly valuable for use in connection with an arc lamp.

Another type of filter, especially valuable when an arc lamp is used in combination with a filter passing ultra-violet rays, is one that will eliminate the objectionable ultra-violet. That is, the two filters should be used in combination.

This is necessary when objectives are not well corrected in the ultra-violet region. Plates sensitive to these short waves record an image in the ultra-violet which is not apparent visually on the ground glass. This ultra-violet image, superimposed on the visual image, lowers the quality of the picture.

Photomicrographers who possess a microspectroscopic eyepiece, a hand spectroscope or more elaborate model, can make good use of it in studying the transmission bands of unusual filter combinations, and stains. The knowledge gained by such study aids materially in obtaining a conception of the relation between the visual appearance of colored objects and their transmission characteristics.

One should not be content with his results until he has completely mastered the use of filters. One rule that is particularly worth remembering is, *never use a more contrasty filter than is absolutely necessary to produce a satisfactory picture.* What is wanted primarily is detail, not contrast.

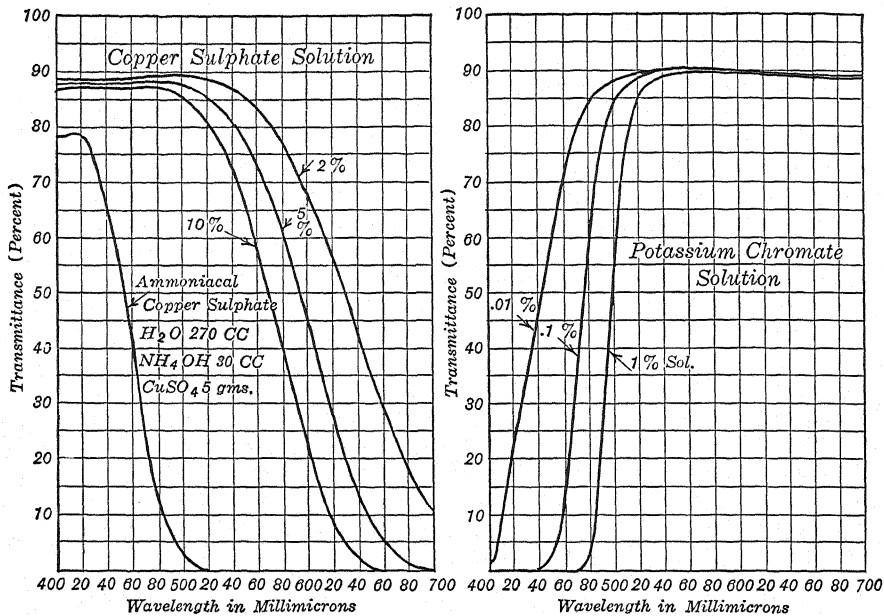


FIG. 89

FIG. 90

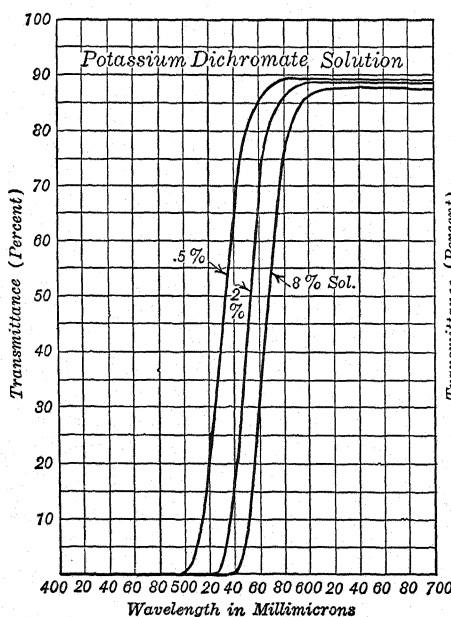


FIG. 91

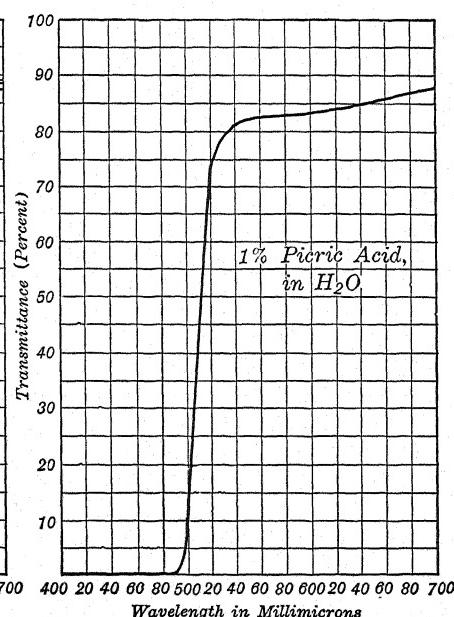


FIG. 92

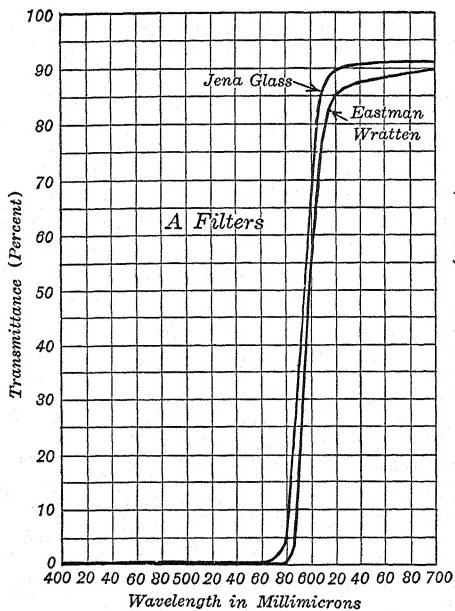


FIG. 93

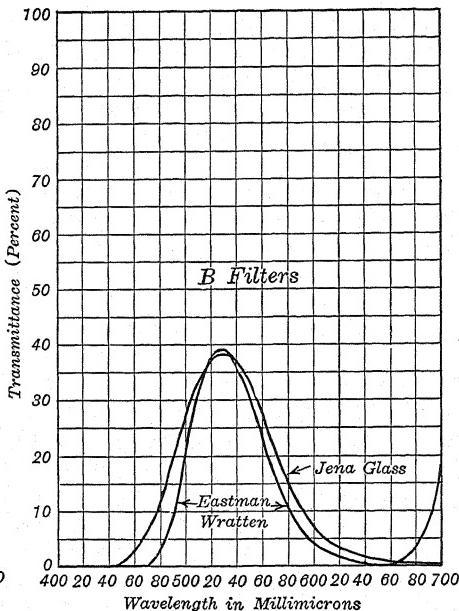


FIG. 94

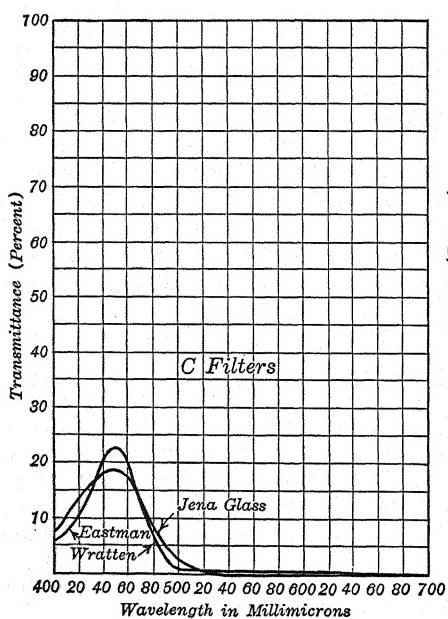


FIG. 95

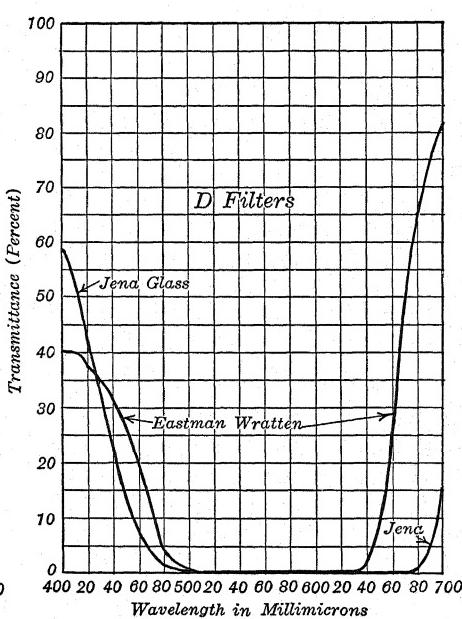


FIG. 96

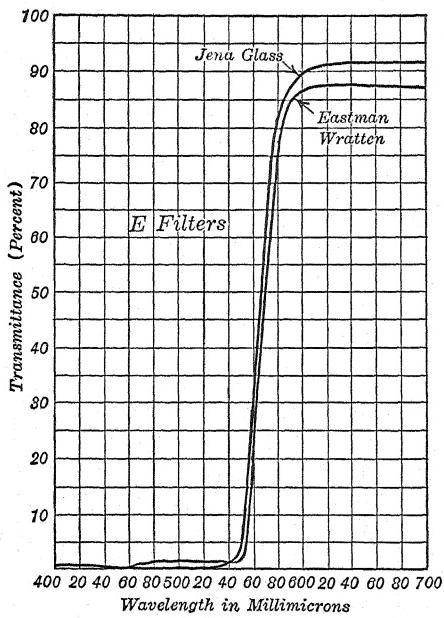


FIG. 97

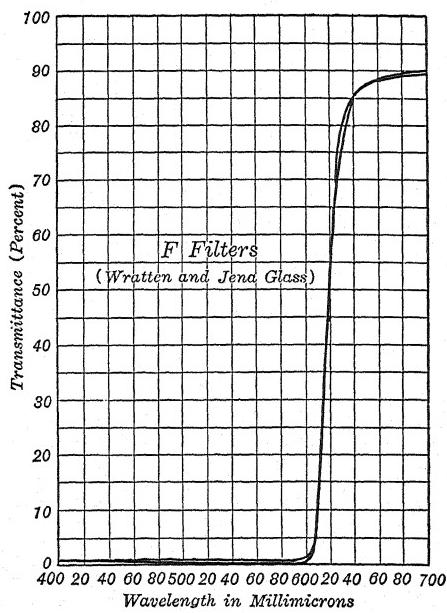


FIG. 98

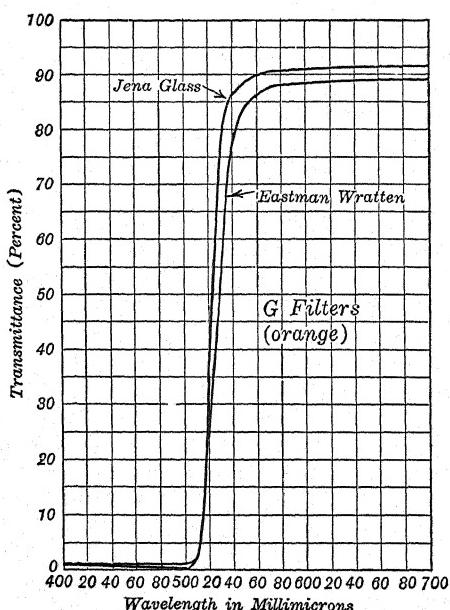


FIG. 99

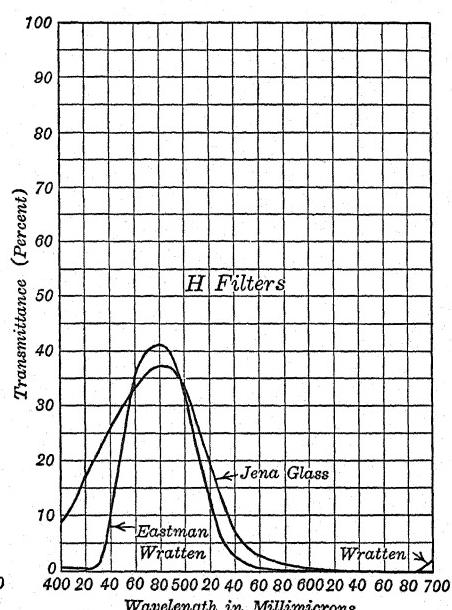


FIG. 100

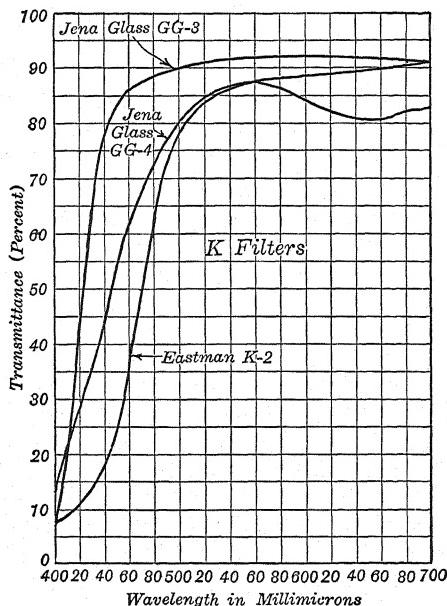


FIG. 101

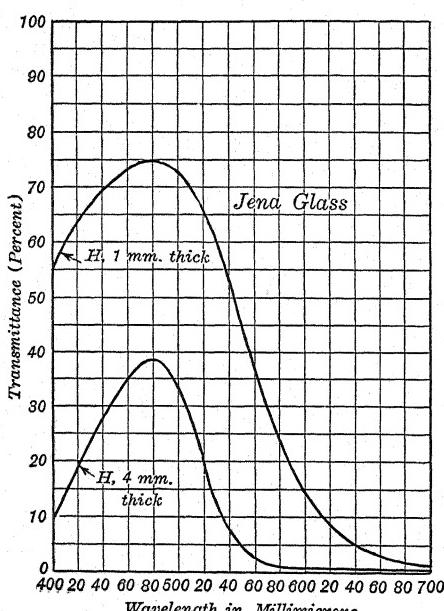


FIG. 102

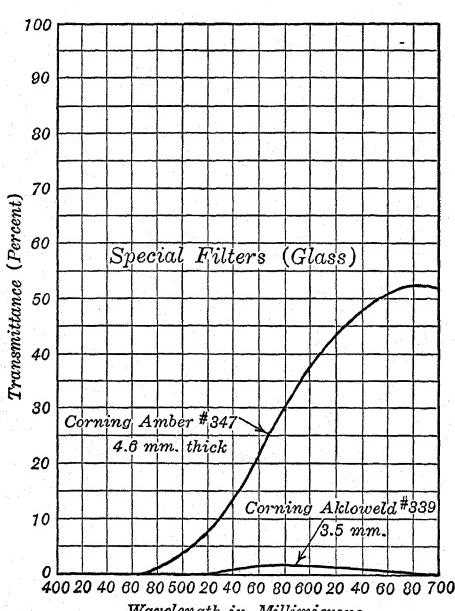


FIG. 103

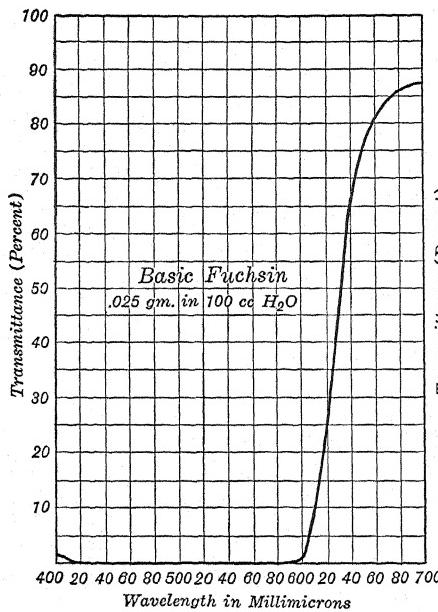


FIG. 104

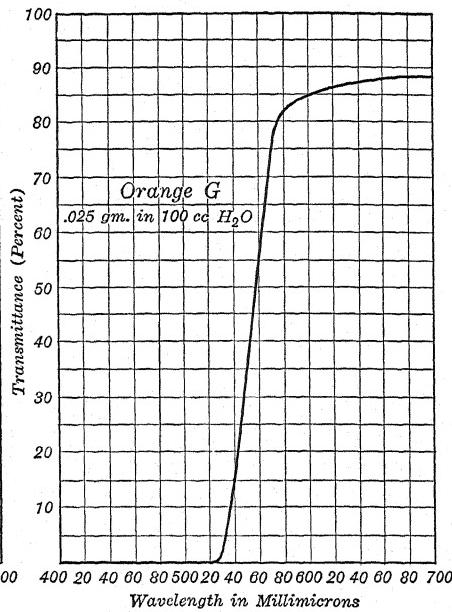


FIG. 105

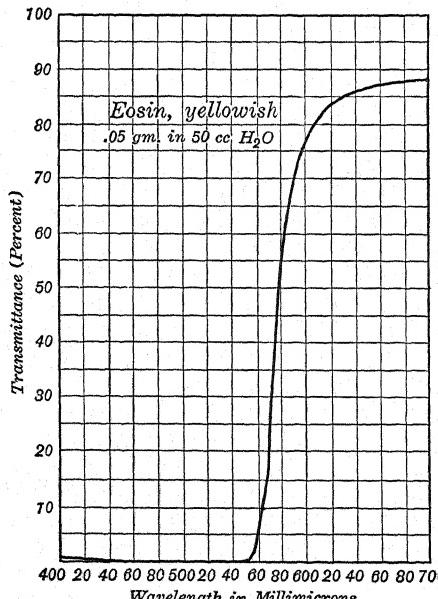


FIG. 106

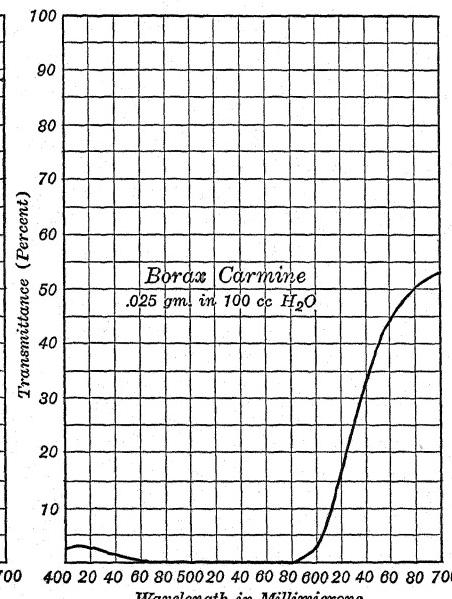


FIG. 107

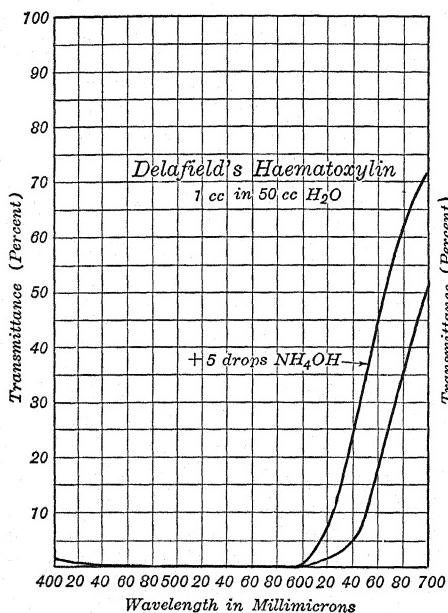


FIG. 108

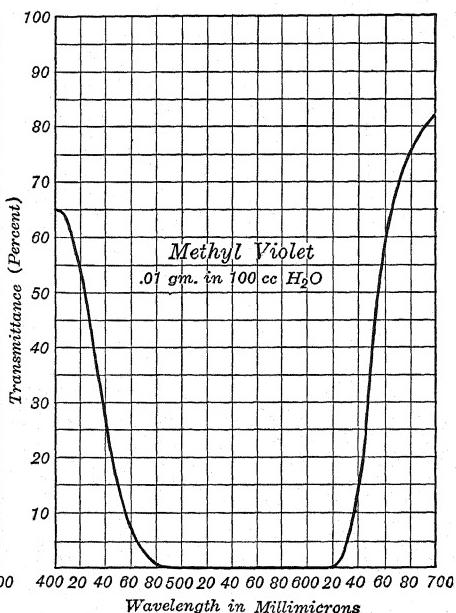


FIG. 109

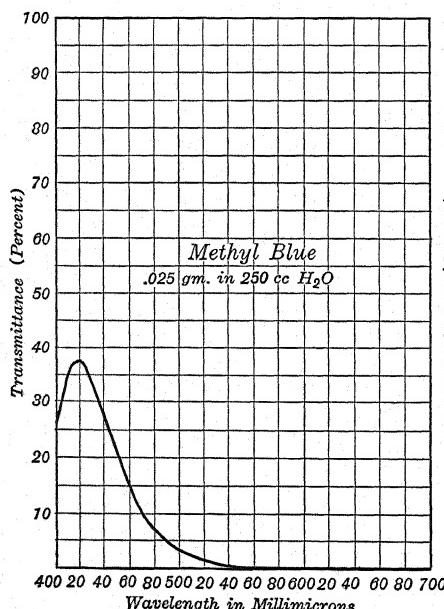


FIG. 110

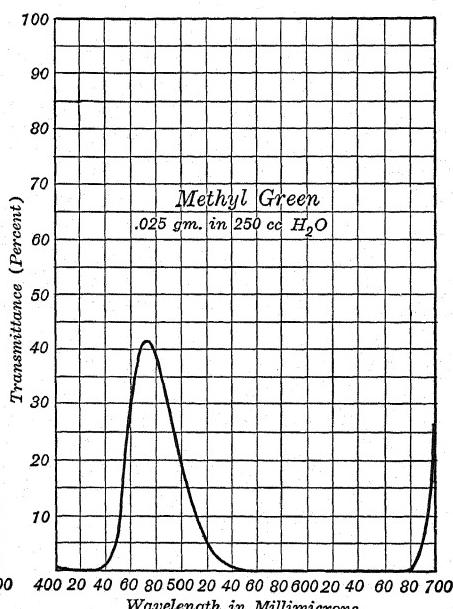


FIG. 111

Taking the Picture

Under this heading we can group the necessary steps in the production of a photomicrograph, from the time an object is placed upon the microscope stage until the negative is ready for development.

Many factors have a bearing on the actual impressing of the image on the sensitized plate. Each of these is so tied up in some manner with the others that they can be conceived of as parts of a whole, rather than as separate matters for consideration. The term "exposure," *per se*, relates to the period of time during which the light is building up a latent image on the sensitized plate. This period of time is influenced by so many variables that it may be of any duration from a small fraction of a second, up to several hours. It therefore becomes necessary to understand in what manner each factor affects the time element.

A. The Factors Influencing the Time of Exposure

The basic principle commonly accepted as governing the exposure time for photographic work is that the intensity of the light and the time during which it acts on a sensitized emulsion are equivalent factors in effecting a definite amount of darkening. For instance, if it has been determined empirically that full blackening of a plate of given sensitivity is produced by 100 units of light energy, this result could be obtained by any number of different combinations. As an example, if the light intensity amounted to 100 units, the time unit for which this light must act would be 1 unit; 50 light units would require twice the time, i.e., 2 units; while a faint light intensity of 1 would require 100 units of time. This is known as the *reciprocity law*.

It has been proved that this law does not always work; it has a substantially straight line function over a considerable range, but beyond this range the line becomes curved. This is called the failure of the reciprocity law. To illustrate, if we continue the case mentioned, $1/10$ of a light unit acting for 1000 units of time should produce the full blackening. When it is tried out experimentally, however, it may be found that only partial blackening has been effected. Indeed, if we lower the intensity of the light far enough, no blackening whatever results regardless of the extent of the time it

might be allowed to act. We have then passed the threshold value of the light for a given plate.

Fortunately, most of the work of the photomicrographer lies on the straight-line portion of the reciprocity law curve, and hence he can figure his exposure times on a scientific basis. Only when a *very* faint light source is involved should compensation be made for failure of the reciprocity law, by increasing the time over what would otherwise be required.

In microscopy, the intensity of the light source is the intensity of the original light *after* it has been subjected to modification by all the various factors which alter its intensity. Most of these factors are of such character that their effect is mathematically calculable after a basic exposure time is once determined. This simplifies our problem somewhat. The micrographer should know just what to expect from each variable. To this end, let us analyze the part they play and how to take account of them.

(1) *The Light Source*

The basic intensity of the light varies with the kind of lamp employed. While the range between the brightest to dimmest of them is great, once a standard exposure has been established for any given light source, it is usually possible to keep within the maximum and minimum intensity of that particular lamp because of an inherent latitude in the sensitized plates. We can thus have every exposure correct, so far as the basic intensity is concerned. Some slight variation can be expected in all forms of electric light, due to voltage changes on the line. Other minor variations are introduced in arc lamps by the length of the arc, resistance of carbons of varying length, and length of time the lamp is in operation, but these are negligible for most purposes. The intensity of tungsten lamps, on the other hand, varies appreciably with changes in voltage, and to a lesser extent with the time a lamp has been lit and the total time it has been in service. Color work is most likely to suffer noticeably when these variations are considerable.

With respect to the light source, the photomicrographer is much luckier than the outdoor photographer who must contend with the radical variations present in daylight.

(2) *The Plate Speed*

Each type of plate has its own speed rating and a basic exposure must be determined for each kind used. It is not likely that the plate speed will change; hence, when a ratio has been established between several brands, it can be used with a fair degree of assurance in determining exposure times, with two exceptions. One is that the failure of the reciprocity law may be different with every type of plate, and the other, that speed ratios will not be constant with all colors of filters. This latter condition, which is the more serious, will be discussed in detail in connection with the effect of filters on exposure time.

(3) *Filter Factors*

The way in which a filter modifies the exposure time can be understood from Figure 112A, which shows the relative amount of light passed by the green filter the curve of which is shown in Figure 94. If no filter were used the entire area included between 0 and 100 per cent transmission and between 400 and 700 millimicrons might represent the total light energy from a given light source, at our command. Assuming that a plate is employed which is sensitive to all rays between 400 and 700 millimicrons, all available light energy would serve to build up an image on the plate when no filter is used. The graph of the sensitivity of such a plate is shown in Figure 112B. Insertion of the green filter cuts down the light efficiency to a degree equal to the ratio of the area of light passed by it, to that passed when no filter is used. If the two areas were as 10:1 then the filter would admit only $1/10$ of the original light energy to the plate. The filter would possess a factor of 10 and whatever exposure would be required without a filter would have to be multiplied by ten to secure an equivalent exposure when the filter is used. Notice that in this case it would be the green rays which would be doing all the work of blackening the plate; but this would make no difference, as the plate chosen is affected by green rays as well as by those of other colors. The same condition obtains with filters of any other color characteristics when used with plates sensitive to the entire visible spectrum. In each case the filter factor would be represented by the ratio of the area of light passed to the area of the whole square.

But suppose a plate were used which was sensitive only to blue light. Such a plate sensitivity would be graphed as in Figure 112C. At once it is apparent that if the green filter (Figure 112A) were inserted in the light train, it would cut out the very portion of light required to affect the blue sensitive plate, while all the light which the filter passed would be of no use in making an exposure for none of it would register on the plate. The filter factor in such case would be extremely high, and in some cases approximate infinity.

It is this condition which makes possible the use of a red lamp in the darkroom when developing plates *not* sensitive to red.

On the basis of the above, two statements can be made as to the effect of filters on the exposure time: (1) *The basic exposure without a filter must be multiplied by the filter factor of whatever one is employed;* and (2) *The factor of any filter will vary with the kind of plate being used.*

To these must be added a third condition which affects the filter factor. So far it has been assumed that the quality of the light from any source is the same, and equal for all wave lengths in the visible spectrum. This is far from being true. Every type of lamp has its own particular curve of intensities for various portions of the spectrum. Figures 112D and E give an approximation of the difference between an arc lamp and tungsten filament lamp. The former is strong in violet and relatively poor in red; the tungsten is just the opposite. This being true, it becomes necessary to add a third effect of filters in the light train, i.e., *the filter factor varies for different types of light sources.*

(4) *The Effect of Magnification*

By means of the illuminating train and substage condenser, a definite intensity of light per unit area is concentrated on the object. This light is employed to produce the image. When the latter is ten times (in linear dimension) the object size, the superficial areas of the image and object are to each other as the *square* of ten is to one, i.e., 100:1. The light, per unit of area, is only 1/100 as great in intensity on the image as on the object because it has been spread over an area one hundred times as great. According to the reciprocity law the time must be multiplied by 100 for a ten-power enlargement, over what would be required to photograph the object at natural size. Increasing the magnification to 20x, i.e., *twice* the previous amount,

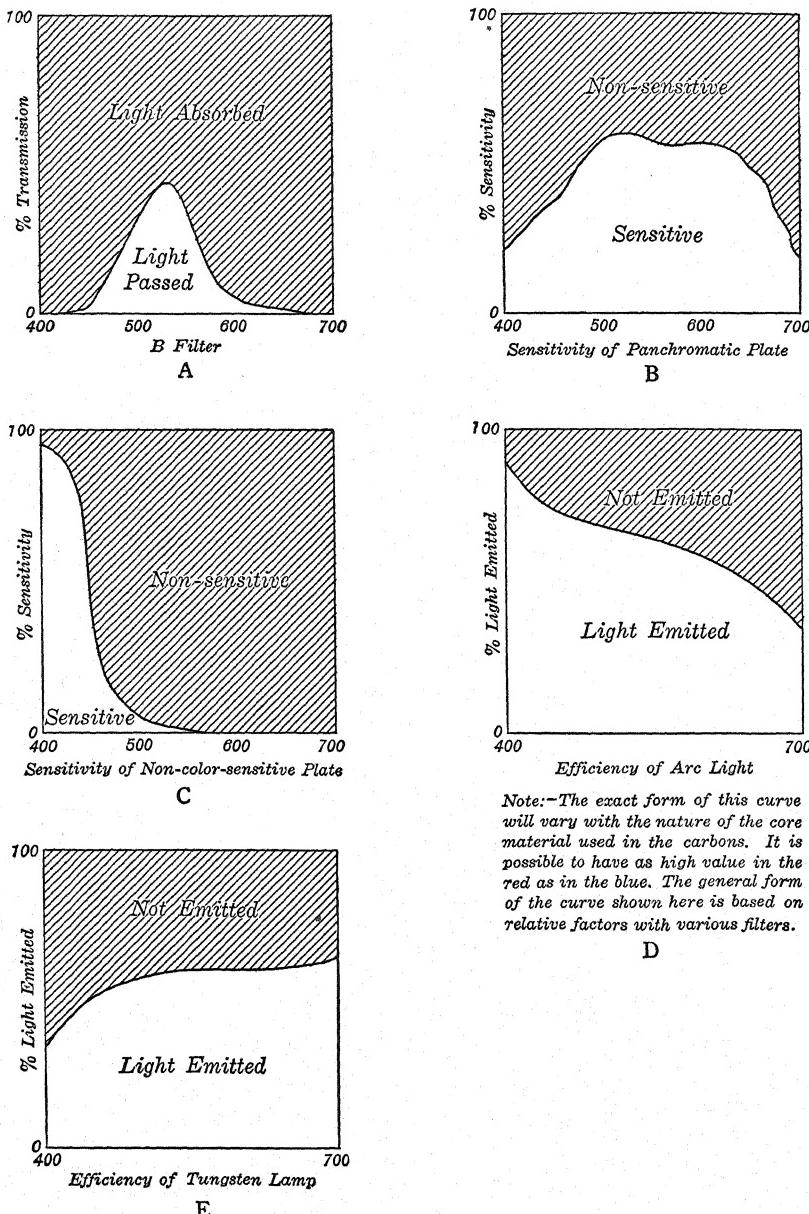


FIG. 112. Curves Showing Color Aspects of Filters, Plates, and Lighting

Note:-The exact form of this curve will vary with the nature of the core material used in the carbons. It is possible to have as high value in the red as in the blue. The general form of the curve shown here is based on relative factors with various filters.

D

decreases the light intensity to $1/400$ the original value. This is only one-quarter as much as obtained with a $10x$ magnification. From this we can formulate the effect of magnification in a general law: *The light intensity varies inversely as the square of the magnification.*

It is important to note that this law, as stated, is independent of the objective, eyepiece, or projection distance, all of which are concerned in the production of the magnification. It makes no difference *how* the magnification is obtained; the only consideration is the *amount* of magnification.

(5) *The Effect of Numerical Aperture*

The numerical aperture of an objective controls not only the resolution of detail in the image, but the light-gathering power of the lens as well. The former increases *directly* with the increase in N.A., but the latter increases as the *square* of the N.A. Assuming other conditions to be constant, the substitution of an objective of *double* the numerical aperture for one of the smaller value intensifies the light *four* times. This enables the reduction in light intensity attendant on high magnification to be canceled out to a considerable extent, through the accompanying increase in N.A. of the higher-power objectives.

This effect of increased light intensity with numerical aperture is subject to one condition — that critical light is employed, the aperture of the condenser (as established by the diaphragm opening) being substantially equal to that of the objective. Obviously, if the aperture of the condenser is reduced, the illumination of the object will be cut down accordingly. The objective cannot gather in light which is not there to be picked up. Should the condenser diaphragm be completely closed, no light would be present. For this reason, the law relating to the effect of numerical aperture on the exposure time must be stated in two parts: *The light intensity varies as the square of the N.A. of the objective, with full critical illumination; with less than full equivalent aperture of the condenser, the light intensity varies as the square of the effective aperture of the condenser.*

This rule applies to low-power photographic lenses as well as to the regular series of objectives.

(6) Effect of Density in the Object

Microscopic objects vary over a wide range in their degree of transparency or opacity. Naturally this is an important factor in determining the exposure time. It is one which is not amenable to a fixed law, other than the general one that the more the light is absorbed by the object, the longer must be the time of exposure. One of the common rules of photography also applies here — that is, *always expose for the shadows and let the high lights take care of themselves*. In photomicrography the object (or certain portions of it) constitute the shadows; the high lights are represented by the clear areas surrounding the object. But these two rules give us no mathematical value which can be inserted into a formula. Relative densities must be determined largely on the basis of experience. For the purpose of establishing a standard exposure value, however, little difficulty need be encountered in the selection of what might be designated a "typical subject." This might be defined as a well-stained tissue (animal or vegetable) which in thin section is transparent and colorless in the unstained condition. Most of these should possess an average density that can serve as a standard with which more dense objects may be compared.

(7) Effect of Aperture Variations in Low-Power Lenses

When a low-power lens, with an aperture, let us say, of $f:4.5$, is used with a long bellows extension its effective aperture approximates the full aperture (which is realized only with a projection distance equal to infinity). As the projection distance is shortened, the front conjugate focus is lengthened and the effective aperture is decreased accordingly. It reached its lowest value (in photomicrography) when the magnification reaches $1x$ (full size). The focal length is then doubled and the aperture is $f:9$. At this aperture the light intensity is only one-quarter of that at $f:4.5$. This alteration in the aperture must not be lost sight of when using low-power lenses for photomicrography.*

* In calculating the time of exposure from a basic value established on numerical aperture (as is naturally the common practice in photomicrography) when using low-power lenses whose speeds are expressed in "f" values and which are equipped with iris diaphragms capable of being set at lower apertures, it is desirable to be able to express the effective aperture being used, in terms of numerical aperture. The nu-

All these factors combined establish the ultimate intensity of the light energy upon which depends the production of the latent image on the plate. They can be stated in terms of a formula which will work out to an answer giving the time required to expose the picture. Before this can be done, however, one value in the equation, a constant, K , must be known. This constant is to be determined empirically. It represents the basic time under a definite setup.

(8) Effect of Foggy Lenses

Glass surfaces which are allowed to stand for some time without being wiped off usually develop an atmospheric fog or smoke. This is not noticeable in ordinary light but an intense beam of light projected on them discloses its presence. For this reason all condenser lens surfaces should be wiped clean whenever they lose their brilliant polished appearance. This fog can affect exposures materially, especially in the blue and violet regions of the spectrum.

B. Determining the Basic Exposure

The basic exposure should be established on a typical slide under conditions of magnification, filter, plate, and lens combination most nearly approximating an average condition for the class of work one

merical aperture equivalent of an "f" value for any given magnification can be determined by means of the formula:

$$\text{N.A.} = \frac{M}{2f \times (M + 1)}$$

This formula automatically takes care of the variable conditions mentioned above, and hence offers the easiest method of introducing the aperture effect into a time exposure computation. For those doing considerable work in the low-power field and using various diaphragm openings, a table of N.A. equivalents for different "f" values at different magnifications can be worked out for ready reference.

Should one desire to know whether a given setup of magnification and "f" opening results in exceeding the theoretical limit of resolution, this can be readily ascertained by the simple expedient of moving the decimal point of the resultant N.A. three places to the right (i.e., multiplying by 1000). This figure should not exceed the magnification, to meet this requirement, although this does not imply that good pictures cannot be taken under the assumed condition, even when the limit is materially exceeded.

It should be apparent that the focal length of the lens does not affect the light-gathering power, and hence it makes no difference in the exposure whether a lens be a 20 mm. or a 100 mm., providing the "f" value is the same in either case.

plans to do. If the magnification range contemplated extends all the way from low powers to very high, an intermediate value would be around 300 \times . The objective required for this combination will be either a 10 \times or 20 \times (40 \times only with very short bellows). Assuming it to be the 10 \times , with the outfit all set for operation, a slide placed on the stage, critical light secured, and the image focussed on the ground glass, a test should be made of the effect of various filters on the appearance of the image. One should be selected which seems to give the best effect visually. Let us assume that this is the green filter (B).

We are now ready to make a test exposure. When a multiplicator back is available the procedure is as follows: Place the multiplicator back in position, put the slot diaphragm in place, then the loaded plate-holder. The number of test exposures which can be made will depend upon the size of the plate and the width of the slot diaphragm used. The 10 mm. slot is ample and provides the largest number of exposures. If there be something of special interest in the specimen, it should be brought into position so that it falls in the area of the slot. Every test exposure will then contain the object of special interest. As the test exposures are made the plateholder is moved along to the next position, and so on until all are taken. Test exposure times are always made in a geometric ratio; starting with the shortest, each succeeding exposure is double the preceding one. For the assumed condition of 300 \times magnification, 10 \times objective and green filter, with a 500-watt tungsten lamp, a safe series can be (in seconds): 1, 2, 4, 8, 15, 30, 60. Should the image be quite faint on the ground glass and the plate not large enough to accommodate so many, the series can be 5, 10, 20, 40, 80 seconds.

Development of the plate will at once show which one of the strips had the correct exposure. Figure 113 shows a print made from such a test plate. The time series in this case was 2½, 5, 10, 20, 40, 80 seconds. Even the 2½ second exposure made a faint impression on the plate, but printing for the strip which was correctly exposed (#4 at 20 seconds) completely blackens both this and the 4-second strip. Accordingly, in making the print, it was progressively exposed, the bottom portion receiving 12 seconds, the top, only 1 second. Only the bottom part of the print shows the 20-second strip as it should be, but at the top is seen the kind of picture resulting from the under-exposed strips. At the right is shown a 12-second print of the #4 strip. Incidentally, this particular exposure test would not be quite representative of that assumed as an average condition. It represents

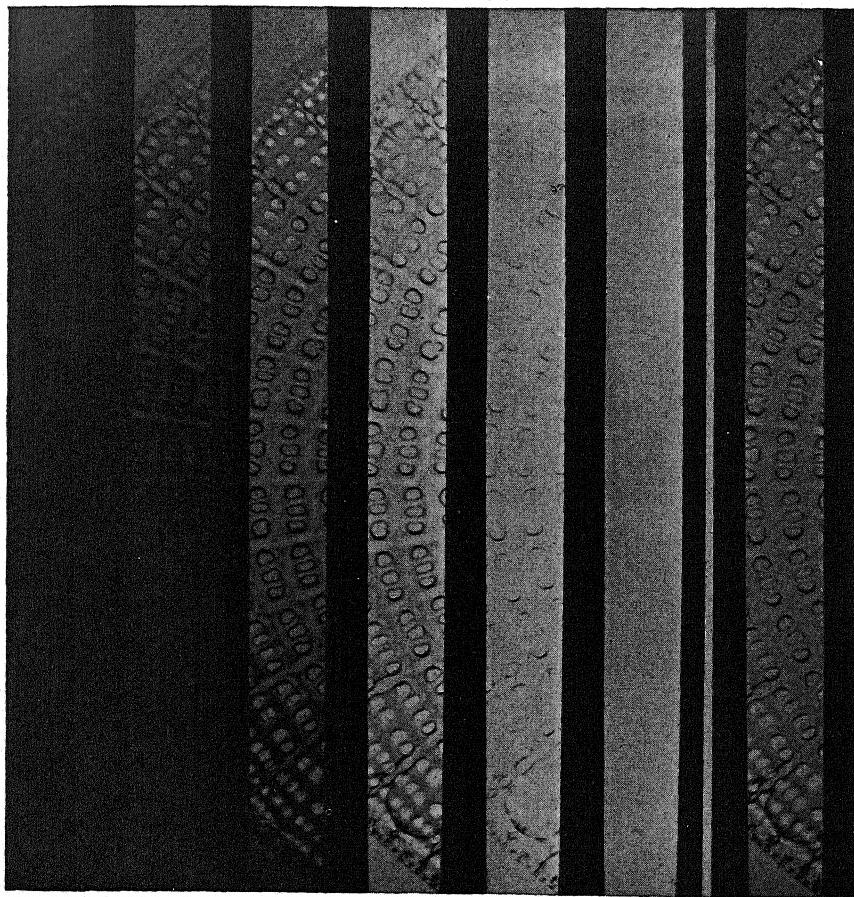


FIG. 113. TEST EXPOSURE TAKEN WITH MULTIPLICATOR BACK

The object, a diatom, *Arachnoidiscus*, at a magnification of 500x, taken on a Hammer Slow plate, the exposure being at $2\frac{1}{2}$, 5, 10, 20, 40, and 80 seconds.

To show the presence of a faint image in the short exposure strips, the print was dodged progressively from one to twelve seconds, only the bottom portion showing the full exposure time. At the extreme right a 12-second print of the 20-second exposure strip is shown, in order to give a correct interpretation of this exposure. A poor picture can be made from the underexposed 10-second strip by printing for one second, while a fairly good print is possible from the 40-second strip by printing for about two minutes.

the type of test that might be made by one whose interest lay in the photographing of diatoms.

Understanding the principle involved in the making of a test ex-

posure will enable anyone to make such test with an ordinary plateholder. Here he does not take a duplication of strips of the same area, but must proportionately expose a portion of the entire field. To do this, the plateholder slide is withdrawn to allow the entire plate to be exposed, but not completely out of the slot. A computation is made as to the number of exposures desired and the length of the plate subdivided accordingly. If the strips are to be, let us say, $\frac{1}{2}$ -inch wide, the first exposure of 1 second is made on the whole plate. Then the slide is pushed in $\frac{1}{2}$ -inch and another 1-second exposure made. The half inch covered up would have received only one second exposure, all the rest of the plate two seconds. The slide is again pushed in $\frac{1}{2}$ an inch and a 2-second exposure made. Similarly after each exposure the slide is pushed in and the following exposure is made, doubling the previous time. Marks can be made on the plateholder slide to indicate its position for each exposure.

Moving the plateholder slide between exposures may result in some displacement of the camera back and the long exposures may be blurred, but this should be ignored.

Similar test exposures should be made for each type of plate used, unless information covering the relative sensitivity of the plates to the type of light used can be secured from the manufacturer. Relative daylight sensitivity is no criterion to follow for photomicrographic work.

When the standard exposure has been determined, it should be recorded, with all other data, as follows:

Standard Exposure — object of normal density

Objective — 10x, N.A. .30

Magnification — 300x

Filter — B (green), Factor 8 times (manufacturer's data)

Plate — Kodak M

Light — 500 watt concentrated filament

Exposure, 30 seconds (this is the constant, K, required)

A data book should be kept for recording all such information. It will become valuable as time goes by.

C. *The Computation of Exposures*

Having determined the standard exposure time, it becomes a simple matter to compute the equivalent time for any other combination.

This is done solely on the basis of substitution of the numerical value of the changed factors.

For instance, let us assume the effect of single changes on the time.

Having found out empirically that the Kodak Panchromatic plate is 3 times faster than the Kodak M, if it be substituted for the latter, the exposure would be

$$30/3 \text{ or } 10 \text{ seconds.}$$

The orange filter (G) has a factor of 2 as compared with 8 for the green filter. Substitution of the orange filter will mean an exposure of

$$30 \times 2/8 \text{ or } 7\frac{1}{2} \text{ seconds.}$$

If we wish to increase the magnification to 400 \times , using the same objective, as the light intensity varies inversely with the square of the magnification, the time required under the new condition would be

$$30 \times \frac{400^2}{300^2} \left(\text{i.e., } \frac{16000}{9000} \right) = 53 \text{ seconds.}$$

Substitution of a 20 \times objective with an aperture of .65 for the 10 \times .30 N.A. will affect the exposure as has been explained, by

$$30 \times \frac{.30^2}{.65^2} \left(\text{i.e., } \frac{.09}{.3925} \right) = 7 \text{ seconds.}$$

Just as it is possible to compute the proper exposure time when only one variable is present, so it can be done even when every one of them is changed. Making all the changes as above at one time the equation becomes

$$\text{K (base exp.)} \times \text{filter factor} \times \text{mag. factor} \times \text{N.A. factor} \times \text{plate factor} = \text{exposure}$$

$$30 \times \frac{2}{8} \times \frac{400^2}{300^2} \times \frac{.30^2}{.65^2} \times \frac{1}{3} = 1 \text{ second.}$$

Should the object be changed to one more dense, the difference in the density must be estimated and this factor also inserted in the equation.

The simplest method of solving the equation is by the use of a slide rule. The relation of the squares can be instantly read off the A scale, when the numbers are set in ratio on the C and D scales, thus saving the trouble of computing them. Inexpensive slide rules are

available; they need not be so accurately engraved as when required for more important determinations.

Where certain fixed combinations are employed to a great extent, data tables can be made up for ready reference. These will save re-computing every time the same set of conditions is present.

(1) *The Use of Light Meters*

The question is frequently asked, "How about the use of light meters?" These have been found so successful in ordinary photography and motion-picture work that they should be of value in photomicrography. In the earlier days of light meters, those available were not sufficiently sensitive to be of much value in photomicrographic work where light intensity is very low. Ordinary light meters are, however, useful when photographing opaque objects at low magnification, from unit size to a few diameters. In using them for this work, where ordinary photographic lenses are usually employed, it is important to take into consideration the corresponding reduction in lens aperture with a short front focal length. When copying full size (one-to-one ratio) the lens aperture is reduced to one-half that at infinity, for which the lens is rated. Thus the light intensity is reduced to one-quarter. Then as the enlargement is increased, the effective light on the focal plane decreases as the square of the magnification. These factors must be taken into account in computing the exposure from the meter reading (see also page 165).

Of recent years meters designed especially for photomicrographic work have become available. The increased sensitivity of these runs from at last twenty times that of ordinary camera meters up to hundreds and even thousands of times.

Photometric measurement of light intensity through the microscope can be made in two ways, either at the exit beam from the eyepiece (the Ramsden circle), in which case the total available light is measured, or at the focal plane where the picture is taken. The latter is open to several objections. In the first place, one would hardly care to invest a considerable amount of money in an extremely sensitive meter to be used only for this purpose, when the less sensitive and less expensive meters will usually serve the purpose, although not satisfactory for focal plane readings. Therefore, in determining the proper meter to purchase, the service required of a meter must be the determining factor.

Again, measurement of the total light impinging on the meter at the Ramsden circle must be considered in relation to the intensity at the focal plane. As the light intensity in this case varies inversely as the square of the diameters, the diameter of the exit pupil where the light is measured and that of the projected image must be known and actual exposures computed on this basis. For example, if the diameter of the former is $\frac{1}{4}$ " and the latter 4", the light value on the film will be $(\frac{1}{16})^2$ or $1/256$ that of the meter reading.

For color pictures this method of determining exposures can be of value since the object (if of normal density) can be removed from the stage to take the reading, and then placed back in position to take the exposure. Allowance must be made for dense objects, but not for color rendering. This cannot be done ordinarily with black and white pictures, where the total light value must be integrated between the light and dark areas. Measurement of the total light impinging on the sensitive film gives no indication of the exposure required for dark areas (often the most important part of the picture) and therefore the human equation must be invoked to determine how the total light should be divided between the light and dark areas.

The ideal location for photometric readings to be taken is at the image plane, where relative exposure times can be determined for each area of the picture. Then the photographic rule for good pictures can be applied, "Take care of the shadows and let the high lights take care of themselves." In other words, expose for the darkness areas, and if necessary take care of overexposure of bright areas by development control. The principal objection to focal-plane readings is that only the very sensitive, and hence the expensive, meters will serve here, unless one is concerned with low-power macrographs where the exposures are in the order of a fraction of a second or at most only a few seconds. Readings for black-and-white pictures should be taken without the interposition of filters, and then the actual exposure determined by the filter factor of the filter that is to be used in taking the picture.

Where meters are available and the photographer is not experienced in photomicrographic work, exposure meters will be found of great value at the start. After experience is gained, the methods described for determining exposures will be found satisfactory, and as is common practice with many camera experts, 100 per cent results can be achieved without the bother of getting out the meter. How-

ever, when estimating exposure time by the appearance of the image on the ground glass, the eye must be allowed to return to normal adjustment after exposure to a brilliant light; otherwise the image will appear darker than it actually is, owing to an excessively contracted pupil.

It is perhaps needless to point out that regardless of the sensitivity of the meter employed, it must be calibrated by the user for work of

different kinds (bright field, dark field, polarized light, opaque and vertical light, metallurgical, work etc.) before it is of practical value.

Several microscope manufacturers supply exposure meters or list them with their accessories. Other companies make a specialty of meters.* Figure 114 shows the Photovolt Model 200M, which does not require batteries or outside current. While this model is especially adapted for reading at the exit pupil, its increased sensitivity of about 20 times that of an ordinary exposure meter allows its use under other conditions where the intensity of the light is high. For those desiring

FIG. 114. A Light Meter. The Photovolt, Model No. 200M.

greatly increased sensitivity for focal-plane readings, other types of meters are available. These utilize photoelectric cells and electronic amplification. Since such cells are not self-generating, electric power must be supplied. They are available for use on a power line (115 volts a.c.) or with enclosed dry batteries. Complete information on these can be secured from the manufacturers.

D. Miscellaneous Items Related to Exposure

There are numerous little details related to some part of the photomicrographic process which do not amount to much in themselves,

* Among these may be cited the Photovolt Corp., 95 Madison Avenue, New York City, and Brinkmann Instruments, 378-80 Great Neck Road, Great Neck, N.Y.



but which in the aggregate will be found to have considerable bearing on one's proficiency in the work. It is necessary to be constantly on the alert for new ideas and means for improving one's photomicrographic technique, through the application of some of the things which experience alone can reveal.

(1) Darkening the Room During Exposure

It has already been pointed out that means should be available for darkening the room where the work is being done. There are two reasons for this. One is the possible interference of extraneous light in the illumination of the object, especially at low magnification. The other is the inconvenience of focussing properly in a brightly lighted room. Although the use of a focussing cloth over the head is possible, it is entirely unnecessary. By the simple expedient of darkening windows and turning off all artificial illumination in the room, focussing and manipulation of the slide can be carried on without discomfort. When this procedure is made a regular habit it will be found decidedly worth while.

(2) Checking Possible Vibration

It may be some time after the photomicrographic apparatus is set up and in use before any evidence of vibration can be detected. This may be due to intermittent conditions, or to a failure to detect the type of vibrations which cause trouble. A lookout for this possible trouble should be maintained from the beginning. Occasional fuzziness of pictures, not traceable to any other cause, should be ample excuse for suspecting vibration. If it seems possible that some is present, one of the best methods of combating it is to place sponge rubber pads 6 or 8 inches square and at least an inch thick under each leg of the table upon which the apparatus is mounted. On top of the rubber pads place similar-sized wood or metal plates to distribute the load. The table legs rest upon the plates.

(3) Securing a Sharp Focus on the Ground Glass

It is extremely difficult for the unaided eye to determine when the image is in perfect focus. For this reason focussing glasses are provided by the manufacturers. The common form is a 6-power mag-

nifier mounted in a tube support which can be rested against the glass. The magnifier must be adjustable to adapt it to the eyes of different individuals. The camera is either provided with a clear glass focussing screen which can be substituted for the ground glass, or a small cover glass is cemented to the center of the ground glass with Canada balsam. This eliminates the ground surface and the glass appears as though clear at this one spot. The eye cannot discern an image on clear glass, but a magnifier picks up the image just as the microscope eyepiece does that of the objective, even though no screen be present.

The clear glass focussing screen, in combination with the magnifier, provides a critical focus, especially with low and medium powers. It is subject to one limitation, however, which has made it responsible for poor pictures in many cases, because of lack of information regarding it. *It is very important that the magnifier be focussed on the glass surface corresponding to the position of the plate.* For long bellows lengths and for medium and high powers it does not matter, but for short projection distances and low-power lenses where eyepieces are not used, this is imperative. To avoid trouble in this respect, the magnifier must be adjusted by focussing sharply on a mark on the ground glass; then it is ready for use with either the ground or clear glass.

When the magnification is high for the aperture being used (i.e., near the point of empty magnification) the focussing glass will work better on the ground glass, because a six times enlargement of an image already on the "empty" side is so fuzzy, it is of no use for determining the correct focus. Under this condition a far better method is to discard the $6x$ magnifier altogether and use an ordinary reading glass of two or three power, on the ground glass. Focussing becomes quite easy under this method.

(4) *The Use of Diaphragm Masks in Front of the Plate*

As a rule, the larger outfits are equipped with metal diaphragm masks which can be placed in the back of the camera, directly in front of the plateholder. The masks are made with different-sized circles, adaptable to various sizes of plates.

For work where the print is preferably finished as a circle to simulate the visual appearance, the mask is valuable in circumscribing the view to the form desired for the print. In this way one can be sure of what is included. But it often happens that a rectangular or square

area is superior in that a higher magnification or a greater area is provided. Under this condition the mask is better omitted. Examples are included in the plates of Chapter 10 illustrating the use of the full length of the plate. Then again, there are occasions when only a circular print is required and the object is a dispersion of small particles. By omitting the circular mask, if the entire plate is covered by the image, a choice of area is offered, from which to make a selection illustrating the material in the best possible manner.

(5) Choosing the Proper Filter

The technical characteristics of filters and their effect on the exposure time have been covered already, but the novice may be more or less bewildered as to how to make a final decision on which to use under a given condition. No one need be ashamed of his ignorance in this respect, for this is a problem likely to stump the best of us. It is true that experience in the taking of many pictures is a material aid in the majority of cases, but it sometimes happens that the color scheme of an object does not seem to be amenable to any possible combination of filters.

Study of a couple simple examples will help to explain the situation. Figure 115 shows three views of a fern rhizome in longitudinal section. These were taken with the multiplicator back, using red, green, and blue filters respectively. The section was stained with fuchsin and methylene blue, the scalariform vessels taking a deep red color; the rest of the cells are predominantly blue but with some residual red from the fuchsin and some greenish areas, caused by reactions between the methylene blue and the cells (the staining being done on fresh-cut sections without previous fixing).

The red filter (i.e., used on the picture on the left) has completely eliminated the scalariform vessels but shows the other cells. The green filter (center) accentuates the red vessels and also brings out the other cells nicely. The blue filter (right) also shows the vessels well but with less contrast than the green. There is enough green and red in the other cells to make them show nicely but with lessened contrast. With this specimen the green filter is obviously the best, for it makes the scalariform vessels stand out better. This does not present any serious problem as to which filter to use.

But now look at Figure 116. It shows the head and beak of the cotton boll weevil, also photographed with the red, green, and blue filters.

Though the insect has been cleared and mounted in balsam, its color is still a rich brown. The red filter reveals the detail structure beautifully; both the green and blue are hopeless. But the red filter is deficient in one respect. *What has become of the beak appendages evident in the other views?* In the green filter picture they show



FIG. 115. LONGITUDINAL SECTION, RHIZOME OF FERN

Three views taken with multiplicator back, showing the results obtained with three different filters. Left — A filter (red); Middle — B filter (green); Right — H filter (blue).

with the proper amount of contrast, but all the other parts are too dark. The trouble lies in the paleness of the color in the appendages as compared with the depth of color in the rest of the body. The appendages are practically invisible in the red light.

Expressed in general terms, it is always a problem when a filter of a certain color must be employed to show detail in one portion of the object, and yet this same filter either renders some other portion

opaque or completely transparent and invisible. Sometimes it requires two pictures to tell the whole story. When this is impossible, a juggling of the exposure time, over- or underexposing, as the case may require, supplemented by over- or underdevelopment, may effect a compromise.

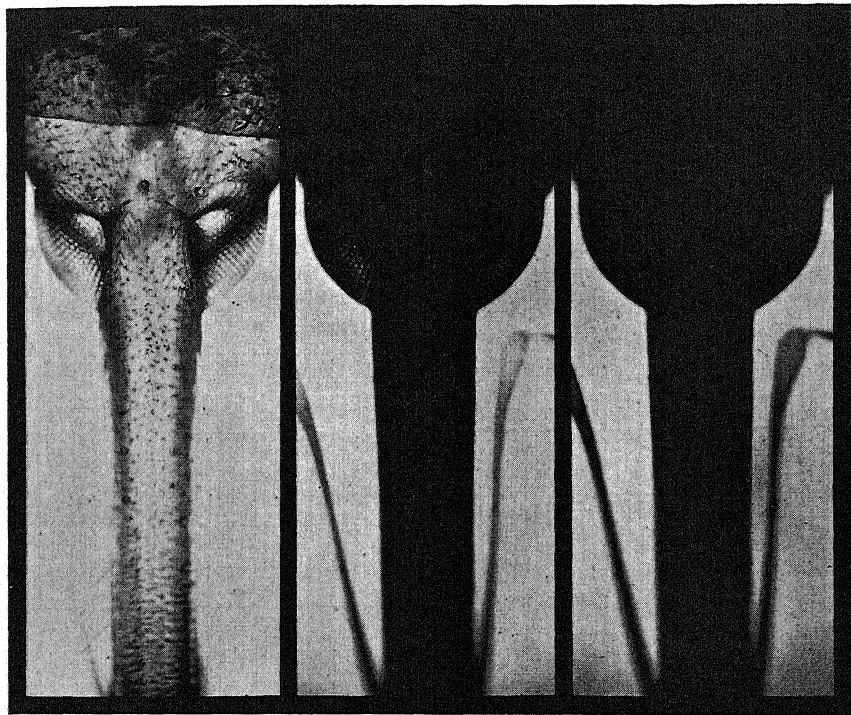


FIG. 116. HEAD AND BEAK OF COTTON BOLL WEEVIL

Three views taken with multiplicator back, showing the results obtained with three different filters. Left — A filter (red); Middle — B filter (green); Right — H filter (blue).

(6) Pictorial Composition in Photomicrography

It may sound a little incongruous to refer to pictorial composition in photomicrography, but there are times when the composition of the subject within the picture area is a matter of importance. No hard and fast rules can be laid down to serve as a guide but attention to this aspect of the photomicrographic problem will usually produce

higher quality pictures. Among the type of questions that should be considered are:

- (1) How much should be included in the picture?
- (2) Where an entire object is to be shown, how much border is required around it?
- (3) Which way shall it be turned, horizontally, vertically, or diagonally?
- (4) In case of a poor area which must be included in the picture (an artifact, torn section, dirt, out of focus portion, etc.), where shall it be located in the picture space, to be least noticeable?
- (5) Where only a segment of a circular object can be included, should the outer portion be located at the top, bottom, or side?
- (6) Where two or three objects are to be included in the field, how should they be placed to convey the impression of balance in the picture?

Many such questions might be cited. An artistic temperament is a great help in giving individuality to one's work. Two adjuncts of the large research microscope aid in obtaining the best answers to these questions. One is the rotating stage, by means of which the object can be turned to different positions to study the effect. The other is a fine-motion mechanical stage. These, combined with an adjustable bellows, and a little patience in examining the image on the ground glass, will usually result in a better picture.

(7) Elimination of Artifacts

To the photomicrographer who desires excellence, one of the most disappointing conditions is the presence of some defect at the very place on the slide where the picture must be taken. Shrinkage of the tissue in fixation is usually not very objectionable, provided no tearing has resulted. The artifacts most objectionable are pieces of dirt, knife lines, torn sections, folded over or buckled sections, and the like. For visual work these are not objectionable; the eye is capable of discounting their presence and concentrating on the point of interest. But a picture is different: by some process of psychology the very thing that shouldn't be seen becomes the center of interest, or, at any rate, detracts attention from the rest of the picture. Elimination of artifacts is not always possible, but numerous expedients may be tried with a view to getting rid of them. Among these are a slight change in magnification to throw them out of the field; decentering the main

point of interest; use of a square or rectangular mask instead of a circular one; or at least placing them in some position in the field where they are least likely to attract attention.

Although retouching, as commonly used with ordinary photographs, is not desirable in photomicrographic work, yet if it is solely for the purpose of eliminating, or rendering less conspicuous, undesirable artifacts, it is justifiable. If one understands retouching, it is usually an easy matter to eliminate knife scratches, tears in tissues, by the use of pencil or knife. Dirt spots can often be eradicated by careful use of a red dye which can be gradually built up until its intensity matches the background, so far as the printing effect is concerned. In the instance of dirt in the balsam surrounding an isolated object, blocking out of the entire background may be the easiest solution. Where the background can be pure white, this is not objectionable.

(8) *Occasional Fuzziness of the Image*

There are several conditions which may produce poor results in a micrograph of an object which appears perfect when examined visually. It is desirable to be cognizant of possible causes of such poor pictures and how they can be eliminated.

Mention has already been made of the effect of foggy lenses on the exposure time, but the condition can also become so serious as to detract from the photographed image. Experts usually can detect any inferiority in the visual image arising from this cause; but even if this has been overlooked, it can show up in the photograph. Recognizing the cause in this case indicates how it can be corrected. There is, however, another condition in which the objective can be at fault. This is a slight efflorescence in the cementing material between a pair of cemented lenses in the interior of the objective. It usually shows up in visual work as a slight fogginess which was not originally present in the lens and which cannot be traced to any other cause. It is frequently difficult to detect by ordinary examination of the lens itself unless one has access to a Greenough binocular microscope. The only cure for this condition is to return the lens to the manufacturer for repair.

Still other factors may be responsible for poor micrographs although the visual image is perfect. One is vibration occurring between the microscope and the camera when a fairly long exposure

is required. A single vibration usually has little effect because of the shortness of the time involved; but should a jarring of the instrument or camera result in a permanent displacement of the image on the film, a double image is formed. Continual vibration can produce the same double image, but usually only a lack of sharpness is evident, or a widening of lines which should be sharp.

Another condition sometimes occurs due to the fine focus of the microscope not remaining fixed. Sluggish response of the fine focus mechanism is responsible for this, especially if the sliding surfaces become gummed up. Oiling and removing the gum deposit is the cure. But in taking long-exposure micrographs a somewhat similar effect is caused by gradual heating of the glass slide, object, or objective, resulting in a change of critical dimensions when high powers are involved and sufficient cooling means have not been provided. Straight Köhler illumination results in more heat and requires correspondingly more coolant to remove it.

When photographing minute particles dispersed in fluid (e.g., fat droplets in milk, fresh blood, etc.) or mounted in a medium which requires a long time to harden, look out for movement during exposure. Horizontal-type cameras cannot be used for this kind of work, nor can inclined stages be employed for visual examination.

E. Some Common and Unusual Problems in Photomicrography

In addition to the common, everyday problems confronting the microscopist in his photographic work, others more or less unusual in nature may arise from time to time. A brief consideration of some of these, and suggestions as to how they can be met, may prove of value. One of the most bothersome is likely to be curvature of the field, which is always present with ordinary eyepieces. When the center of the field is in focus, the outer margins are all blurred; when the focus is altered to make the outer zones sharp, the center is no longer in focus. Pictures taken under either condition are not satisfying.

(1) Overcoming Curvature of the Field

One worker who took up photomicrography told me of his discovery that everything could not be sharply in focus at the same time. He had laid it to the achromatic objectives he was using and purchased a set of apochromats and compensating eyepieces. His next discovery

was that the trouble, instead of being corrected, was worse. It was then that he sought my advice. He was right in his second discovery; the trouble is usually worse with apochromats than with the less expensive lenses. Conditions are not improved by the use of projection eyepieces, for these also have a curved field.

There are, however, several methods by which fairly flat fields can be secured. First, one may employ a long bellows extension with a low- or medium-power eyepiece. The entire field is then quite large in diameter, and only the central portion of it is used. If one lacks a long bellows, a high-powered eyepiece will accomplish nearly the same result. Combining both is better yet. These methods introduce some complications, in that the magnification is increased at the same time. To lower the magnification will probably require the use of a lower-power objective with a lower numerical aperture. This in turn lowers the resolution and may even bring one into the realm of empty magnification.

Sometimes a compromise can be made by a partial correction through these means, supplemented by a focus on a point midway between the center and circumference of the field.

Most manufacturers now make a series of so-called flat-field oculars for visual work which are superior to the regular series of Huygenian eyepieces. These can be used to some advantage for securing flatter fields in photomicrography, but they are still not perfect. By far the best solution lies in the use of eyepieces such as the Homals, which have been developed especially for photographic purposes.* The field produced by these is beautifully flat. Figures 117 and 118 illustrate the difference in the appearance of the same field when taken with an ordinary compensating ocular and with a Homal. The only fly in the ointment for some, in this solution of the problem, lies in the high cost of the set of eyepieces required to cover the complete range of objectives.

(2) *Optical Sectioning*

One of the most valuable assets of photomicrography lies in the possibility of portraying a single plane in a transparent object of appreciable thickness, without serious interference from layers above and below. This is known as optical sectioning. The only requisite

* Homals are useless for visual purposes, since the eyepoint lies *within* the eyepiece, and not above it, as in the case of ordinary eyepieces.

for its accomplishment is the employment of an objective of high numerical aperture. It is not always necessary to use the very highest possible aperture; it must be adapted to the extent of depth permissible with a given object. In every case the aperture of the substage con-



FIG. 117. GLAND CELLS IN INTESTINE OF NECTURUS

Taken with a compensating ocular, and showing the curved field characteristic of all ordinary eyepieces. Only the central portion of the field is in focus.

denser must also be high, preferably corresponding to that of the objective, and the light must be critical. If desired, a series of optical sections can be made showing progressive changes in structure. For further visualization of the structure, it is possible to make prints of

each picture in a group of serial sections, on lantern slides, as transparencies. These, when piled together in proper sequence, provide a beautiful three-dimensional effect. For the depth to correspond accurately with the magnification the distance between the progressive

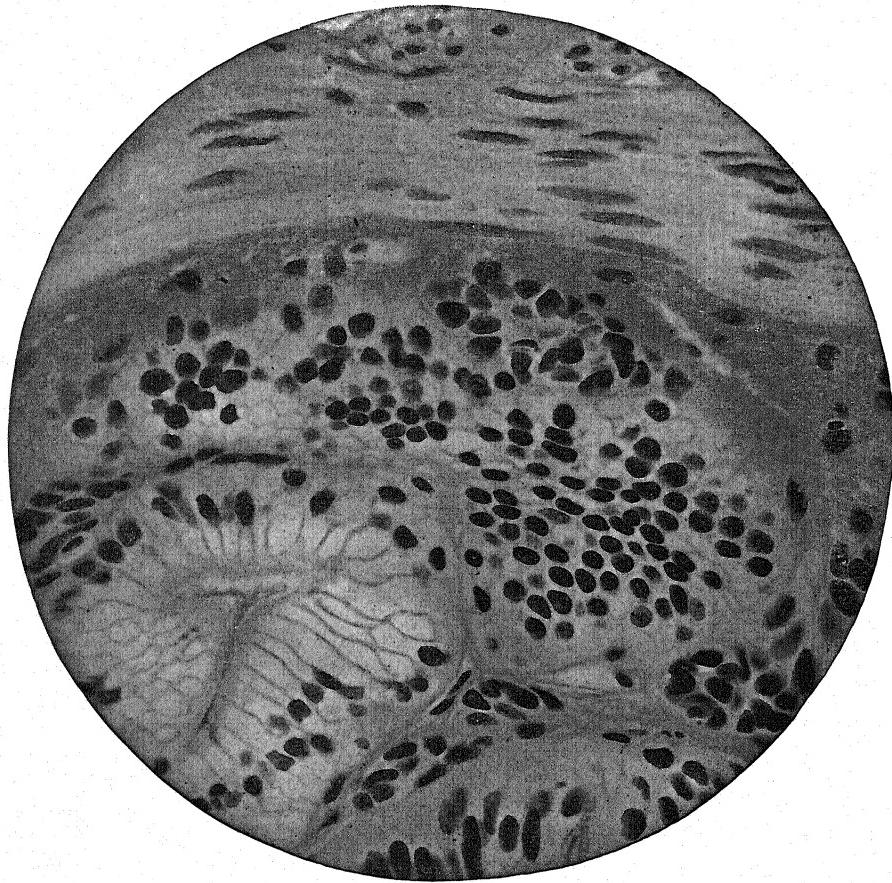


FIG. 118

A view of the Same Subject as in Figure 117. Taken with a photographic eyepiece (Homal) which gives a flat field over the entire area

steps in the series should be computed so that it is related to the thickness of the glass of the transparencies in the same ratio as the surface enlargement. For instance, if a magnification of $1000\times$ is employed, change in depth of each view should be $1/1000$ of the

thickness of the transparency glass. In measuring the movement on the graduated fine adjustment of the microscope, to secure this actual depth, the refractive index of the medium in which the specimen is mounted must be taken into account. This is usually around 1.5, hence if the desired thickness be 1.2 microns, the registered movement must be $\frac{1.2}{1.5}$ or .8 micron.* The transparency prints must be rather light if many levels are to be superimposed.

(3) Securing Depth of Focus

This is exactly the opposite of optical sectioning. The manner of its accomplishment therefore lies in doing just the opposite of what one would do for optical sectioning — that is, use lenses of the lowest possible numerical aperture. Magnification must be secured by high-power eyepieces and long bellows extensions. Reduction of the sub-stage condenser aperture can also be resorted to, up to the limit where diffraction effects begin to intrude.

Though many conditions in transmitted light work call for the greatest possible depth of focus, this requirement is still more common for opaque objects with very uneven surfaces.

(4) Overcoming Conditions in the Object

One cannot photograph many slides without discovering the existence of numerous conditions which are detrimental to ideal results. Sections are found which are wavy and cannot be got in focus everywhere at once. Some sections are cut very thick; others are stained too darkly, or hardly at all. The glass slips used for mounting are often wavy and uneven to the extent that specimens such as blood smears, bacteria, etc., are not everywhere in focus at the same time at the very point where a particular field is wanted. Some unstained objects possess a refractive index almost identical with that of the media in which they are mounted. These and others of like nature are continually cropping up and each may require the employment of new techniques, or modifications of old. In general, familiarity with the fundamentals already discussed will suggest suitable methods to meet such situations as they arise.

* Only stands with the very finest of fine adjustments can be expected to meet this small movement.

Wavy sections in histological and pathological work are extremely common, and sometimes tax the ingenuity of the photomicrographer to the limit. The novice may recognize that something is wrong, without being able to detect the cause of the trouble. When one part of the field is in focus, another is out. The focus can be changed to bring the latter in sharply; then the former is fuzzy. Several methods of eliminating the trouble are possible, depending upon the amount of leeway one has, in the magnification, area required, etc. Such devices as raising the magnification to reduce the area of the field; shifting the area slightly; and occasionally using an eyepiece with a curved field which compensates for the curved section, are all possibilities. Using a lower-aperture objective, as described above, for securing depth of focus, is one of the best methods. This necessitates high eyepiecing or increased projection distance to restore the magnification to the desired amount. Uneven slides cause trouble only in high-power work with bacteria, blood, and similar surface smears. The effect is identical with that produced by wavy sections and the methods of overcoming it are the same. Sometimes two or more cells are desired to be in focus at the same time, yet a high-aperture objective is required. In this case, one or the other of the cells must be sacrificed.

Thick sections can be handled in either of two ways, depending upon the result desired. One is to use a low-aperture lens with considerable depth of focus to get everything in the section sharp. This is usually the better method for low- and medium-power work. When high power is permissible, or required, the optical section method will solve the problem but care must be taken to focus on the most important plane in the section.

Dark objects and sections that are intensely stained must be given ample exposure, supplemented, where possible, by the use of a filter which will reduce contrasts to a satisfactory degree.

Unstained, colorless objects mounted in media having refractive indices close to that of the objects, require a reduction in the aperture of the cone of illumination to produce the necessary contrast. Such objects are said to possess low relief, or low refractive index differentiation. Critical illumination with a full cone from the condenser must be modified in order to introduce diffraction. Although diffraction is objectionable, as a general proposition, we must allow it here, or not obtain a picture at all.

Figure 119 illustrates the appearance of a diatom (*Auliscus*) at a

magnification of 1000 \times , under three conditions of illumination. The three views were taken with a multiplicator back and the only difference in the conditions was in the aperture of the illuminating cone. The first view (at left) shows the result obtained with a full cone, corresponding to the aperture of the objective. In the second

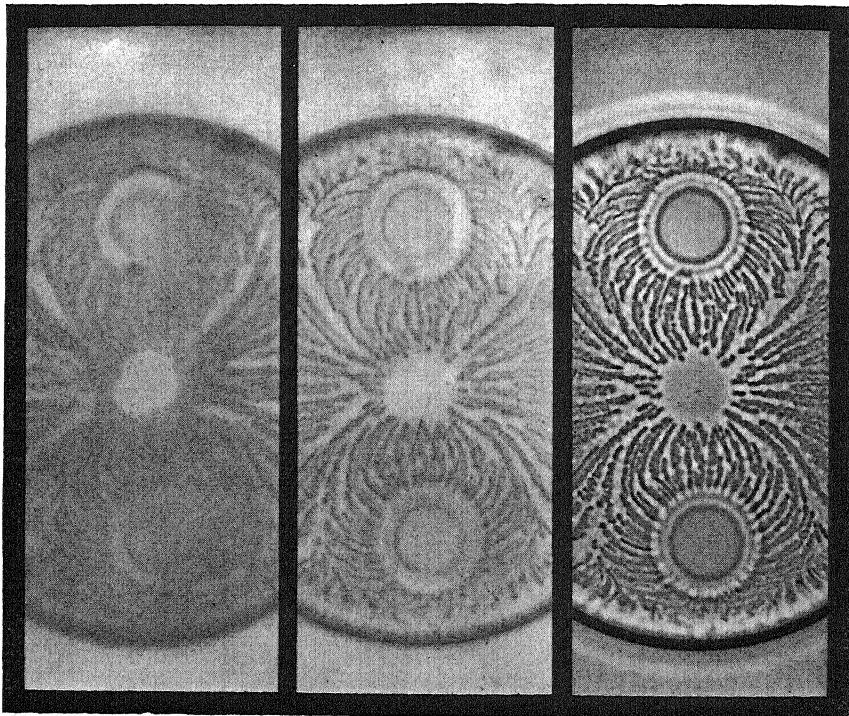


FIG. 119. DELINEATION OF DIATOM STRUCTURE. ACCOMPLISHED THROUGH REDUCED APERTURE OF ILLUMINATION CONE

Left — Full aperture, equal to that of objective.

Middle — Aperture reduced one-half.

Right — Aperture reduced to one-quarter of objective.

view the aperture was closed to one-half that of the objective, and in the third the aperture was one-half of the second. In order to compensate for the change in light intensity, the second picture was given an exposure of four times the first and the third was increased to sixteen times the first. The progressive increase in the contrast is marked, although one condition has not been changed — the structure-

less parts of the diatom are not darkened. They still possess the same tone as the background.

It should be pointed out that whereas the stopping down of the condenser affected the exposure time in the ratios of 1:4:16, the resolution does not suffer to this extent. The resolution is reduced directly as the effective aperture of the system is changed. With a full cone of illumination the aperture is $\frac{1 + 1}{2}$, or unity (i.e., that of the objective in use). In the second case it is $\frac{1 + \frac{1}{2}}{2} = .75$, while in the third it is $\frac{1 + \frac{1}{4}}{2} = .625$.

In ordinary microscopical work it helps materially when one is familiar with the relationship existing between the refractive index of the specimen under examination and that of the medium in which it is mounted. This subject is discussed at length in Chapter 6, where it is closely related to phase microscopy; but it has an important bearing on the question of overcoming conditions in the object which can be overcome in the mounting of it on the slide.

One method of eliminating the effect of refractive index which is almost universally employed in biological, histological, and pathological work is to stain the specimen with suitable dyes. But many materials cannot be stained at all; others can be studied without staining provided they are properly mounted. It is here that a knowledge of the part played by refractive index is important to the microscopist.

It is only by means of the differential existing between the refractive index of the object and that of the medium in which it is mounted that an object can be delineated. That is, if the object is colorless and transparent and possesses an index identical with that of the medium, it cannot be seen at all. It becomes increasingly evident only as the difference between the two indices increases.

Two possible conditions exist: either the object has the higher index or the medium has the higher. As it makes no difference in the delineation of the object which of these is true, the microscopist has two choices in mounting an object. Ordinarily the preferred method is to have the higher index in the medium, for several reasons. The index of many materials lies within a rather narrow range of 1.45 to 1.60. There are few mountants suitable for permanent mounts having indices lower than 1.45, air being the best when the object

can be mounted dry. A serious objection to dry mounts, especially for such objects as diatoms, is that there being an air layer present, the numerical aperture which can be supplied by the condenser is limited to 1.00; there is no advantage gained by oiling the condenser, and maximum resolution cannot be obtained for high powers.

Diatoms are the opal form of silica, with an index around 1.44, and unless mounted dry, they must have the benefit of a mounting medium of higher index. Several are available. Balsam, with an index of around 1.53, is satisfactory for large coarse forms of diatoms and for many materials (hairs, fibers, etc.) which have an appreciably lower index, as well as for mineral powders and the like which have a much higher index. Styrax, another similar resin of vegetable origin, has an index around 1.62. Synthetic resin mounting media up to 1.65 are on the market. (It must be understood that rated media, such as those mentioned, are dissolved in volatile solvents — xylol, benzol, toluol, etc. — and do not reach their maximum index until most of the solvent has evaporated.) A very popular medium, Hyrax, was put on the market many years ago, with an index reputed to be around 1.80, but that figure was probably an error and the Hyrax now available has a stated index of 1.65. Some of the old material in my possession tests at 1.65 in the solvent. Dried out it should go somewhat higher, possibly to 1.70. It is decidedly different from that now supplied. All of these mountants are suitable for permanent mounts.

When only temporary mounts are required, it is possible to employ media of any range desired from N_d 1.35 to 2.11. The Cargille Index Liquids* are eminently suited for use with modern microscope stages which remain in the horizontal position in use, and for photomicrography with vertical-type cameras. (Being nondrying, they are not satisfactory for permanent mounts.) They also serve another useful purpose, that for which they are primarily intended, that is, to determine the refractive index of unknown materials. This is done by putting the material, the index of which is to be determined, into the various liquids until one is found in which it becomes invisible. The index of this medium corresponds to that of the material. To accomplish this it is necessary to interpret the evidence indicating whether a liquid used for test has an index higher or lower than the

* The R. P. Cargille Laboratories, Inc., 117 Liberty Street, New York 6, N.Y. They also supply a permanent mounting medium of 1.65.

object in it. The simplest test, and one that is satisfactory for rough determinations, is that known as the Becke Line Test.

With a minute object in a medium having a slightly lower index than that of the object, raising the focus above the correct focus causes a bright line to appear *within* the border of the object. Upon lowering the focus the bright line travels out of the object into the medium, i.e., it appears to surround the object on the outside. But if the object has the lower index, the condition is reversed, the bright

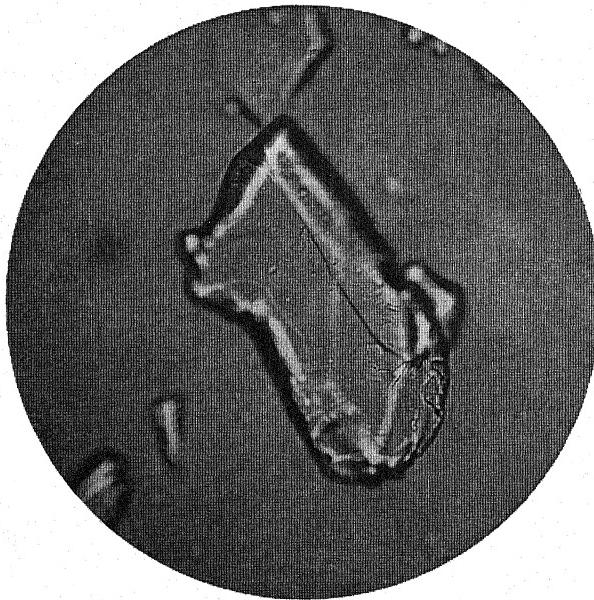


FIG. 120. Glass (100-mesh) with a Refractive Index of 1.58 in Balsam with a Refractive Index of 1.53. Magnification 150 \times . The bright *Becke line* falls inside the particle when the focus is raised slightly above correct focus

line surrounds the object as the focus is raised and travels into the object upon lowering the focus. Figures 120 and 121 illustrate the effect produced by *raising* the focus with two samples of 100-mesh glass particles having an index of 1.58, one mounted in balsam (refractive index 1.53), the other in a medium with a refractive index of 1.65. In both cases lowering the focus will also move the bright line — from inside to outside or outside to inside, as the case may be. In Figure 119 the view on the right side can be seen to have a faint

white border surrounding the diatom. As in this slide the diatom has the lower index (i.e., 1.44 in 1.53 balsam) we know that the focus was slightly raised in order to delineate it to the best advantage. This is frequently desirable to accentuate the contrast (see page 190).

Thus we have a sure means of knowing when an object is mounted in a medium slightly lower or higher in index, and what liquid to try next to bring them closer together; or if it is desired to increase

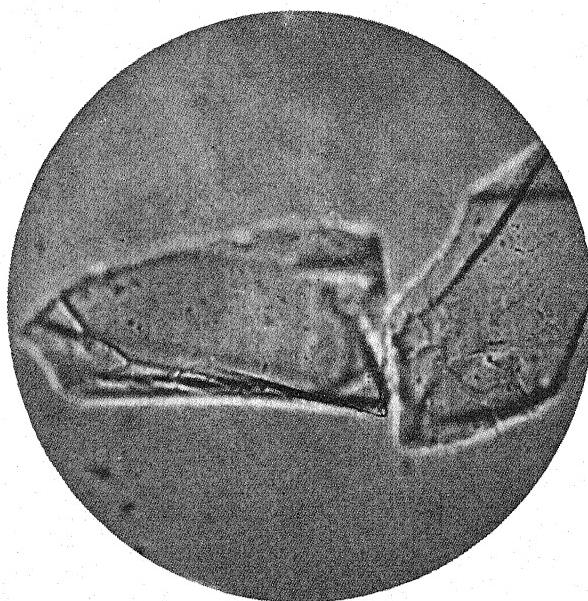


FIG. 121. Glass (100-mesh) with a Refractive Index of 1.58 in a Medium with a Refractive Index of 1.65. Magnification 150x. The bright *Becke line* falls outside the particle when the focus is raised slightly above correct focus

the differential between object and medium in order to increase the contrast, what medium will do the trick.

The number of materials which can be examined and photographed under ideal conditions without being stained is legion — textile fibers, wools, animal hairs, minerals, chemical crystals, starches, pigments, and the like.

Figure 122 illustrates a beautiful result obtained by mounting 2-denier nylon filaments in a 1.65 medium. Since nylon, which is birefringent (indices of 1.52 and 1.58) is so near to balsam (index 1.53), the appearance when mounted in the latter is poor indeed, un-

less examined by phase microscopy. Another example showing the results obtained by mounting a colorless subject in a high-index mountant can be seen in Plate LIII, Chapter 10. Starches, unless they

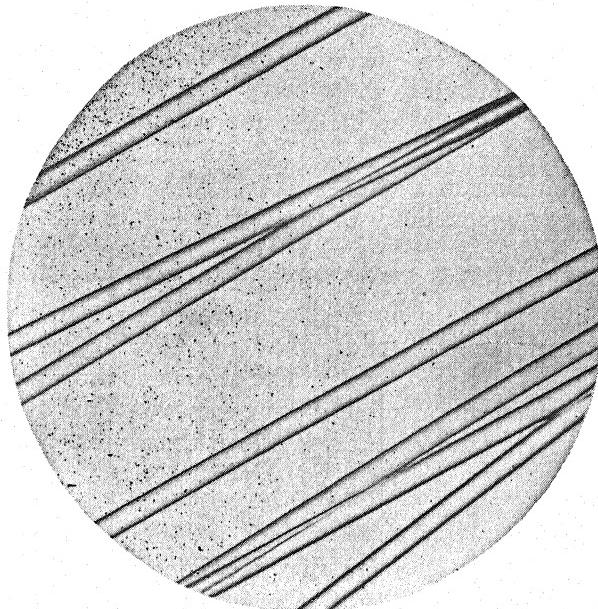


FIG. 122. Nylon Filaments (2-Denier) in a Mounting Medium with a Refractive Index of 1.65. Magnification 200 \times . Note the ample contrast and freedom from diffraction effects that are possible when there is a proper difference in refractive index between the object and the mounting medium

have large-sized grains, show very poorly in balsam, but stand out conspicuously in a 1.65 medium.

(5) *Inherent Limiting Conditions in Photomicrography*

In commercial photomicrographic work, one is frequently asked to meet impossible conditions in producing a picture. For instance, three requirements will be set: a magnification of a definite size, a definite field to be included, and the picture to be made on a 3 $\frac{1}{4}$ " x 4" lantern slide, for lecture purposes. Such requirements are subject to the operation of a law analogous to the phase rule. Any two conditions can be fixed, but the third must be variable. Suppose the stipulated requirement to be that an object 1 mm. in diameter be photographed

as 1000 \times on a lantern slide plate. One millimeter multiplied by 1000 is 1000 mm. or about 40 inches. Therefore, if the object and magnification are definitely fixed, the final picture will be $3\frac{1}{3}$ feet in diameter, which obviously cannot go on a $3\frac{1}{4}'' \times 4''$ plate. Either the magnification or the part of the object included in the picture must be reduced to about $1/12$ the stipulated dimension if the most important requirement is that the picture *must* be shown as a lantern slide. It is sometimes hard to make a non-scientific-minded client see this point.

Another limitation, not often appreciated by microscopists themselves, lies in the impossibility of photographing, in one view, a large object of considerable depth and at the same time revealing minute structure which requires a high-aperture lens to resolve. Here the limiting conditions are diametrically opposed to each other. One cannot have great depth of focus, a low magnification, and great resolution at one and the same time. The answer is usually easy, however — take two pictures. One picture can show the general view, the other the specific detail characteristic of the object.

(6) *Sectional Map Pictures*

It was implied in section (5) that it is impossible to give a client what he may require in the way of magnification for a given object size, provided no limit is placed upon the size of the picture. Naturally the response to this is that the camera limits the plate size, and that therefore a forty-inch-diameter picture is just as impossible as the other requirement. This is true, if we are to be limited to *one* exposure. But just as the aerial photographer can map a whole country by a series of overlapping views which can subsequently be matched and combined into one composite whole, so the photomicrographer can produce map pictures of any size by successive exposures which can be matched together the same way. The author has made numerous pictures of this type, extending to several feet in diameter and composed in some cases of upward of a hundred separate exposures. To do this requires an accurately graduated mechanical stage. A careful layout of the entire object must be made, the size of the included square of the field at the determined magnification computed, and the settings on the verniers of the stage recorded for every square.

The light and all other conditions must be constant, and the ex-

posures and developing conditions on every plate be identical. Some overlap should be allowed on the squares to take care of possible inaccuracy in the setting of the verniers; otherwise one might find a thin strip of area missing at some place. The pictures must match, both horizontally and vertically. It requires only mechanical skill to assemble the pictures. This can be done by means of adhesive strips on the back, the whole being mounted on a large card as the final operation.

(7) *The Use of Extremely High Magnifications*

There is a fascination, for some individuals, at least, in the production of pictures in the realm of super-power magnifications. It probably results from a desire to see and reveal structures that have been beyond the ken of others. Usually nothing results from such pictures and they are only mediocre in quality; nevertheless, there is a distinct value in encouraging this class of work, for in the end we may derive something out of it which will further the cause of science. Some notable work has been done along this line by several workers.

The limiting factors in high-power work are two: the basic optical laws and the perfection of the mechanical and optical parts of the microscope and equipment.

We have seen (Chapter 1) that there is a limit in effective magnification, beyond which further increase results in "empty magnification." This limit is established at roughly 1000 times the numerical aperture of the objective, based upon a circle of confusion of 250 microns.

Some have even insisted that 250 microns is too large a value; that the ultimate limit should be set as low as 75 microns. This low figure, based upon the existence of a few hypothetical individuals possessing abnormally good eyesight, is logically absurd, in the light of actual practice, for it is equivalent to saying that a 1.4 N.A. apochromat, our highest available aperture, cannot be expected to perform, at the strictest standard of useful definition, *beyond a maximum magnification of 420x!*

It is not that there is anything wrong with the theory; the arbitrary standard of excellence is merely set too high. The same criticism applies, although to a far smaller degree, to the 250 micron limit for the circle of confusion, which establishes a maximum useful magnification for a 1.4 N.A. aplanat, at 1400 diameters.

The trouble with the entire resolution formula, so far as it applies to the maximum *useful* magnification, is that it does not take into account the limitation inherent in the human eye, *outside the area of acute vision*.

When we say that the eye can resolve two lines at a certain minimum spacing, when they are located ten inches from the eye, we mean that they can be resolved at the point of best vision, the *fovea*. Away from this area, vision is relatively poor and lines many times more widely separated cannot be resolved. This is the reason the eye does not relish being worked continually at the limit of vision but prefers an ample margin over it.

For instance, if one examines two editions of the same book, alike except that larger type is used in one than in the other, he does not say of the latter, "I do not like this edition because I cannot read the fine print," but he says of the other, "I prefer this one because of the larger type."

Just so with a photomicrograph all the detail may be present in one at $1400x$ which is procurable, but it may be so fine that much of the finer structure is unnoticed. Enlarge the same picture by two, and it becomes a pleasure to look at it.

To illustrate, Plate XI (page 386) shows a section through the cochlea of a guinea pig. It was taken with a Planar, stopped down to $f:6.3$. The magnification is $25x$, slightly more than would be permissible with a 75-micron limit on the circle of confusion. To be sure, the picture is beautifully sharp, but can it, at this magnification, tell us all about the structure shown *which the lens used is capable of revealing*, or as a matter of fact, actually *has* put into the negative for us to see?

The convolutions of the cochlea have a bridgelike membrane through them supporting the organ of Corti, the nerve element which picks up sound vibrations and converts them into energy for transmission to the brain. There is also a second delicate membrane (the membrane of Reissner) extending across the convolutions. These can be seen in the micrograph, but the best human eye known to science cannot learn much concerning the structural details of the membranes, from a superficial examination of Plate XI.

The best way to answer the question raised is to make an enlargement directly from the negative of Plate XI. Figure 123 shows such an enlargement, *seven times actual size*, resulting in a total magnification of $175x$! This enlargement is so great that the grain of the plate is evident and detracts from what the lens itself would produce at the

same magnification. Obviously there is considerable empty magnification present, but the net result is a greatly enhanced appreciation of the structure. The individual cells are discernible with ease.

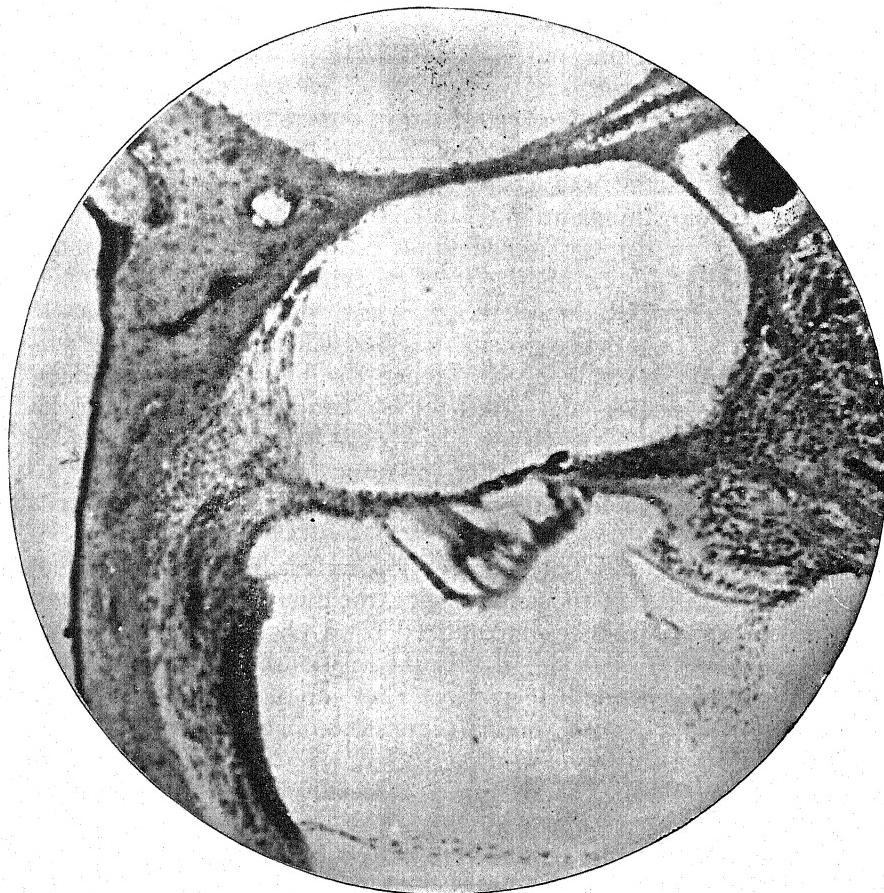


FIG. 123. SEVEN TIMES ENLARGEMENT FROM NEGATIVE OF PLATE XI

Showing a marked increase in the structural details revealed, regardless of the considerable empty magnification and plate grain introduced. The ultimate magnification is 175 diameters.

Although this illustration is based on a low-power lens, the same principle applies all along the line to the very highest-power objective. It is apparent that there are two ways of looking at the question of the maximum useful magnification provided by any given lens.

There is the theoretical limit, which can be stated in mathematical terms, and the practical limit, which cares nothing about theory but is interested only in results. We must therefore disregard all attempts to place a limit on ultimate magnification on the basis of theory, and establish an independent standard.

For practical working purposes, it is a safe law to follow, that *the effective limit of magnification with any given lens is that where the image ceases to reveal additional detail in a manner easily discernible to the normal eye, at the distance of best vision.*

Such a limit extends the working range of objectives considerably. Study of many of the plates in Chapter 10 will show the practical application of magnifications exceeding the theoretical limit of resolution.

To take high-power micrographs requires, first of all, the best quality of objectives; but modern lenses, as turned out by all reputable manufacturers, have more possibilities built into them than are utilized by average microscopists. Hence the limitations imposed on the photomicrographer are often set by conditions other than the objective.

It is necessary, for utilization of the utmost in resolving power, to oil the condenser when using immersion objectives having apertures in excess of 1.0 N.A.

Among the factors influencing results are freedom from vibration, the stability of the fine-focussing device, the duration of the exposure, and the variation in focus introduced by a change in temperature during the exposure. A variation in the relationship between the object and the lens, of one-half micron ($1/50,000$ of an inch) during an exposure, is sufficient to spoil a picture completely, when a high-aperture lens is being used.

Failure to obtain the results desired, especially when the picture does not appear to measure up to the quality of the image as seen on the ground glass, can usually be traced to one or the other of these factors.

(8) *Superimposing Eyepiece Scales and Rulings*

It is frequently desirable to superimpose a net ruling, pointer, or engraved scale on the photomicrograph for showing dimensions, particle size, or designated areas. The one place in the optical system where this superposition is possible is in the plane of the eyepiece diaphragm. A micrometer eyepiece is designed to accommodate any scale which is to be seen at the same time as the object. It is pro-

vided with a focussing eyelens which serves to bring the scale and image in focus simultaneously. In photography it is important to note one condition — when the scale is in focus for visual work it is *not* in focus for the picture. Moreover, the focus of the scale varies with every change in projection distance. Therefore, where using a superimposed scale, the first thing to be done, after establishing the projection distance, is to focus the scale until the lines are sharp, *before* focussing the image.

(9) *Photographing Objects Mounted in Fluid*

Unusual conditions are often present when objects mounted in or suspended in fluids must be photographed. A microscope in the vertical position is essential. This generally implies a vertical camera.

Sometimes, with minute particles, Brownian movement may be present. This requires a fractional-second exposure to stop motion. A more frequent trouble arises when an immersion lens is being used, because the viscosity of the immersion oil exceeds that of the fluid. Every attempt to focus on a fine object results in a movement of the object, because of varying pressure on the cover glass. Under these conditions it is usually preferable to employ high dry objectives, which should be of the type that are adjustable for cover glass thickness.

Low-Power Photomicrography by Transmitted Light

For convenience, the line of demarcation between medium- and low-power photomicrography can be set at the point where single photographic lenses must be substituted for a low-power objective and eyepiece.

Though the fundamental principles applicable to photomicrography in general are not different in the low-power field, there are a few special problems introduced by the changeover that warrant consideration.

In the first place, it is at this point that the Köhler method of illumination for low-power work should be introduced, as constant standards for exposure can then be set up and maintained throughout the entire series of low-power photomicrographic lenses.

To apply this method of illumination, each lens must have its own condenser. This condenser has the same, or substantially the same,

focal length as the photographic lens, for it is located directly beneath the object slide, and its focal point should lie in the plane of the diaphragm of the lens. The diameter of the condenser is the limiting diameter of the field that can be covered, providing the hole through the microscope stage is ample — which is not always the case when the longest-focus lenses are being used. Köhler low-power illumination requires the use of a diaphragm located between the lamp condenser and the substage condenser. The lamp condenser is focussed upon this diaphragm so that the rays cross at this point. This diaphragm is the aperture diaphragm and it should always be open sufficiently to obviate diffraction effects, while for maximum resolution it should provide a full cone, equal to the N.A. of the photographic lens, to the object. This can be determined by looking at the rear of the photographic lens, exactly as with regular objectives.

If all lenses in the entire series have the same aperture (*f* ratio), when a suitable diaphragm aperture has been established for one lens, it can be maintained for all, and changing lenses to provide the desired magnification does not affect the exposure time, in so far as the aperture is concerned. The exposure time, however, is subject to the law relating to the effect of magnification, just as with high-power work. In addition, as has already been pointed out (page 168), exposure times are effected by the variation in *effective* aperture, resulting from any change in the projection distance.

Although it is possible to use the Köhler setup without the interposition of a parallelizing auxiliary condenser between the aperture diaphragm and substage condenser, it is advantageous to employ it. The efficiency of the illumination system is raised by its use, as it is then possible to direct all the rays into the substage condenser.

For low-power work it is imperative to employ a shutter with accurately controlled fractional-second speeds, as very short exposures are the rule, especially when high-intensity lamps are used. It is desirable to make a series of test exposures on a low-power basis, similar to that described for high-power work. In this connection it must be remembered that any change in the size of opening in the aperture diaphragm affects the exposure inversely as the square of the aperture diameter. For instance, if the established standard opening has been set at 20 mm. and for some purpose it is closed to 10 mm. the exposure time must be increased four times. Opening it to 30 mm. from 20 mm. reduces the exposure to $4/9$ of the standardized time.

For low-power work with transmitted light, the iris diaphragm in

the photographic lens (assuming it is equipped with one) should not be materially closed. When not forcing the lens to its limit in magnification, it is permissible to close the diaphragm slightly, as a partial control on exposure times.

For extremely large objects — e.g., whole brain sections — which must be photographed by transmitted light, the microscope is omitted and the lenses are mounted directly on the camera lens board. Then some means must be available for supporting the object and illuminating it uniformly. An opal or ground glass placed some distance behind the object and suitably illuminated from the rear will suffice. Under this condition, filters must be attached to the front of the photographic lens, as in ordinary camera work, and the filters must be of such optical quality that they do not affect the performance of the lens.

With these exceptions, low-power photomicrography with transmitted light does not offer problems in technique radically different from higher-power work.

Photomicrography with Incident Light

The photography of small opaque objects at either low or high magnification by means of incident (often called indirect, or reflected) light has more in common with ordinary photography than has transmitted-light photomicrography.

Two types of lighting are employed for opaque objects. In one type, the light is projected from the side against a reflector located back of the objective. This reflector turns the course of the rays along the optic axis into the back lens of the objective, which becomes, in effect, a condenser, concentrating the light on the object. Such illumination is known as vertical, or specular, illumination. It is employed especially for metallurgical work, from low to the very highest magnifications. This application of it will be discussed in Chapter 5.

Vertical illumination is of little practical use in other lines of photomicrography, because it does not cast shadows. Everything therefore is shown in flat lighting, devoid of contrast, except where strong differences in the absorption of light are present in various portions of an object. Those attempting to employ vertical illumination for general photography of opaque objects invariably are disappointed in the results they achieve.

The other form of incident lighting is usually called oblique top

illumination. With ordinary photomicrographic equipment, it is confined to objectives possessing a considerable working distance between the front lens and the object, so that a beam of light can be projected at an angle, past the objective, to the surface of the object. For this reason, it is largely limited to medium- and low-power work. Magnification up to 50 diameters can be obtained with short-focus microphotographic lenses, and this includes most of the opaque work that is done.

For the range between 50 \times and 100 \times , low-power objectives and high-power oculars can be used successfully, if suitable means of illumination are available.

When the distance between the front lens and the object is short, illumination by a single beam of light becomes impractical because

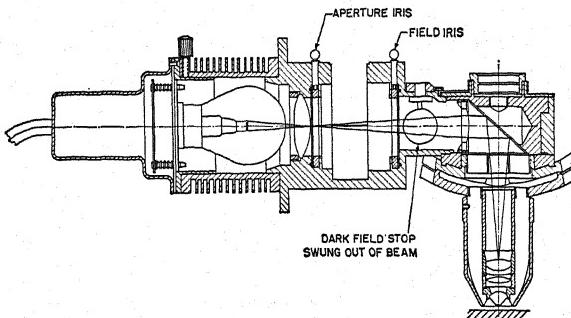


FIG. 124. Schematic diagram of the Bausch & Lomb Tri-Vert Illuminator Adjusted for Bright Field Work

the angle of incidence is so great that excessive shadows are cast and only high spots on the object are illuminated. To obviate this condition various types of illuminators have been developed. They all are based upon the principle of illuminating the object by means of a cone of light more or less surrounding the objective. Among the more modern pieces of equipment for this purpose are the Leitz Ultropak and the Zeiss Epi-W condenser. These employ special objectives and an illuminating system which projects the light around the outside of the objective proper, but inside of an auxiliary shell, directly focussed by reflecting surfaces onto the object.

A recent addition to these is Bausch & Lomb's Tri-Vert illuminator which operates on the same general principle. Figures 124 and 125 show diagrammatically the path of light through the Tri-Vert illuminator when employed for vertical and indirect light uses.

All of these outfits perform beautifully for visual work, but the light supplied is hardly adequate for photography by indirect light unless exceptionally long exposures are used. With the Zeiss Epi-W illuminator, I have found the solution in substituting for the lamp with which it is equipped a 500-watt Ostram lamp, or for high-power work an arc lamp, combined with a condenser system which approximates the path of rays from the lamp of the standard outfit. Plate XLVII illustrates what can be accomplished by this method, even at a magnification of 1000 diameters.*

The value of this type of illuminator for general visual work with opaque objects cannot be overemphasized. Its value begins where the Greenough binocular leaves off, and where it is possible with binocular eyepieces (as with the Zeiss model) to employ half-moon dia-

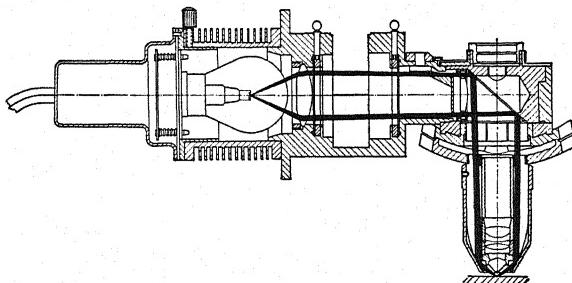


FIG. 125. Schematic diagram of the Bausch & Lomb Tri-Vert Illuminator Adjusted for Incident Illumination

phragms at the Ramsden circle, good stereoscopic effects can be obtained.

One of the commonest problems of photography by indirect illumination lies in securing a considerable depth of focus. Most opaque objects are very uneven in their surface contours and it is necessary to define sharply the low portions, as well as the high parts. This is a case where stopping down the lens aperture by means of the iris diaphragm produces the desired result.

* For the benefit of any who might wish to employ an external light source, the condenser system consists of a hemispherical condenser of as large a diameter as can be used, mounted (plane surface toward the light) in a sliding sleeve which fits into the lamp housing in place of the regular lamp. Parallel rays are projected into it from the light source by means of a collimating condenser, and the hemispherical condenser is focussed at the point where the standard lamp filament is located. While this has not been tried out with either the Leitz or Bausch & Lomb illuminators, it should work equally well with them.

The problem of obtaining just the proper amount of high lights and shadows with microscopic objects is analogous to the same problem with gross objects, portraits, etc., in ordinary photography.

Rotation of the object on the stage; changing the angle of the light beam; use of a second supplemental light; diffusion of a straight beam by means of ground glass interposed; re-reflecting a portion of the light back on the object from the opposite side, are some of the methods which should be tried to obtain the desired result.

In general, when the image looks good on the ground glass, it should make a good picture. It is well to remember, however, that contrasts between light and shadow are apt to be accentuated in a picture, so that it is usually better to work on the flat side, visually, in order that the final results will not possess an undue amount of contrast.

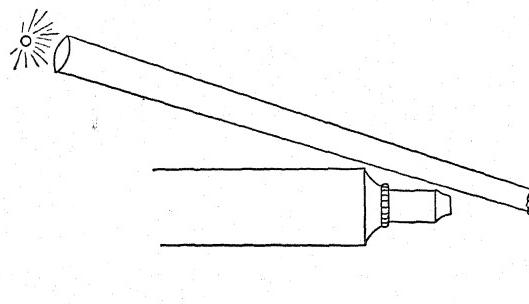


FIG. 126. Spot Light Illumination, with Arc Lamp

No one type of illumination will take care of all opaque subjects, at all possible magnifications. Three general systems will be found useful under different conditions. These are :

(1) *Unbalanced diffused light.* This is secured by the use of from two to several tungsten lights of an intensity suitable to their proximity to the object and the extent of magnification. This lighting is useful only in the low magnification ranges and with fairly large objects. The unbalance is secured either by a variation in the intensity of the lamps on opposite sides, or in the distances of the lamps from the object. This form of photomicrography does not differ from ordinary commercial and portrait photography, except that enlarged instead of reduced images are obtained.

(2) *Spot lighting* with light from a single source. This type of illumination is especially valuable for slightly higher magnifications in

cases where there is ample room to project the light along the side of the objective. The method is shown diagrammatically in Figure 126. For the higher powers, which require an intense light, an arc lamp with a focussing condenser is essential. Such a lamp, mounted on a floor stand (Figure 60) which can be adjusted as to height and position, is ideal. For very delicate objects, which might be damaged by heat, it will be necessary in addition to mount a water cooling cell in the beam, near the lamp. This method of illumination is also useful in low-power work where objects of large area and little surface relief are

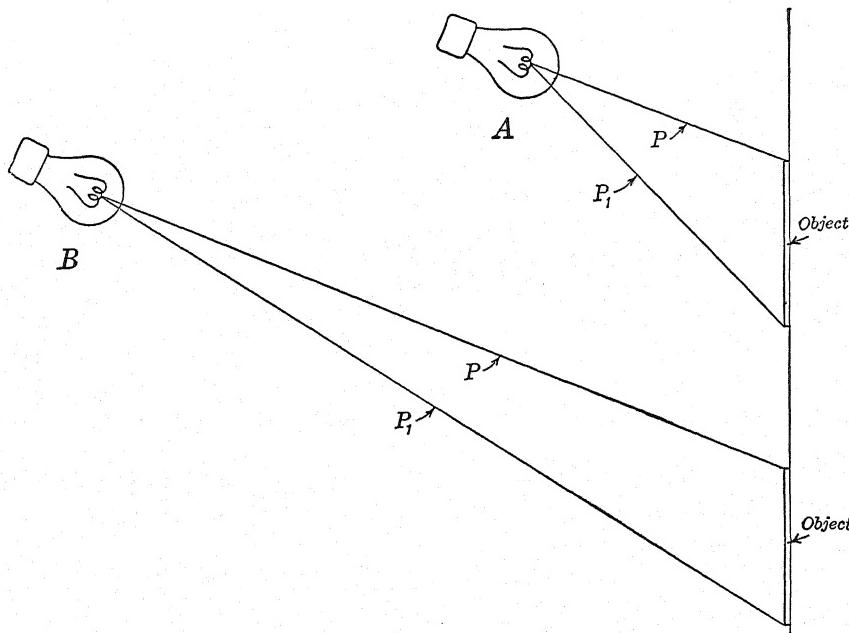


FIG. 127. Method of Securing Even Illumination on Both Sides of Picture

involved. Rather than employ an arc lamp here, however, it is better to change to a tungsten lamp as a high-intensity arc is not required; neither is a focussed beam. One essential in this method of lighting is the securing of even illumination over the entire area. This is accomplished by placing the lamp sufficiently far away to reduce the difference in the lengths of the light paths to the two opposite sides of the object to a small value. Figures 127A and B illustrate the reason for this. At A the relative lengths of the paths (P and P_1) of the rays are to each other as 3:4. The intensity of the illumination varies inversely

as the square of the distance, and hence one side of the object is illuminated by 16 units of light as against the 9 units which the distant side receives. This is nearly a two to one ratio, and an uneven picture is sure to result. At *B* the paths are to each other as 10:11, the light intensity relationship is as 100:121 (i.e., as 5:6). This difference ordinarily is not sufficient to cause an objectionable variation in the intensity on the finished print.

(3) *Side illumination*, deflected onto the object. This method is particularly applicable to higher magnifications, where the working distance between the object and lens is at a minimum, although it can be used for low-power work as well, if the necessary reflectors are available.

For high-power work an arc lamp with a focussing condenser is employed to project a beam at right angles to the optic axis, between the lens and object. This beam is deflected to the object in any one of several ways. Figures 128*A* and *B* illustrate some of the possibilities in this method. In *A*, *m* is a small mirror, plain or concave, as required, which reflects the light, after passing the object, back onto it at a suitable angle. This may be used alone or in combination with a lightly frosted ground glass (*g*) which diffuses the light and softens the shadows. An opposite arrangement is shown in Figure 128*B*. *P* is a small 90° prism located in the path of the rays, deflecting them down on the object. When counter illumination is desired with this arrangement, the size of the light beam must be such that not all of it passes through the prism. That which does not is reflected from the far side, preferably by means of a dead white surface, back to the object. With these general suggestions before him, the practical photomicrographer can devise means to meet any problem. For low-power work, when this system is employed, mirrors or reflectors of a size commensurate with the area to be covered must be used. The light beam itself should also be of large diameter.

One occasionally has to photograph, by incident light, objects which have strongly reflecting surfaces. These cause objectionable high lights in the picture. As reflected light is always more or less polarized, these reflections can be subdued and even completely eliminated by the use of polarizing films such as Polaroid. Ordinarily one such film, mounted over the front of the lens and rotated to the position of extinction for the reflected rays, will be ample. For severe cases it may be desirable to employ a polarizer in the path of the illuminat-

ing rays, also. Any effect desired may be obtained by rotating both prisms until the combination is satisfactory.

It is only occasionally that filters other than yellow or orange are used with opaque objects. Whatever is required can be inserted in

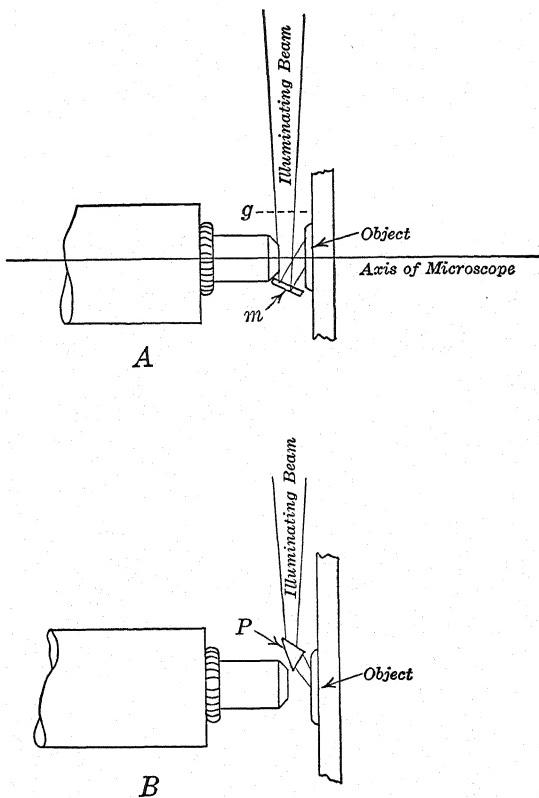


FIG. 128. Side Illumination by an Arc Light

the light train, if the filter size is sufficient. Otherwise they can be mounted on the front of the lens.

It is hardly necessary to add that the time of exposure with opaque illumination is greatly increased over that required with transmitted light at similar magnifications. The best method, if much work is to be done along this line, is to make test exposures, just as recommended for transmitted light.

Common Faults in Photomicrography

What are the faults most likely to be detected in the work of the beginner in photomicrography? An answer to this question may be of material aid in helping to eliminate them. Although the faults should be self-evident from the information given covering methods for the production of high-quality pictures, an analysis of them may put the emphasis where it is most needed.

We can divide the causes of low-quality work into the following groups:

(1) *Faults attributable to poor apparatus, poor optical equipment, and poor focussing.* One should not be hasty in ascribing failures to the quality of the equipment, but the possibility should not be overlooked. Pictures falling into this class are characterized by a lack of sharpness, even when the magnification is well within the range where no empty magnification should be present. It may be due to poor focussing only. It is impossible to focus accurately on the ground glass without using some form of magnifier. This is the first condition to be suspected if non-sharp pictures are encountered.

If the trouble lies in the equipment, the actual cause may be any one of several conditions, each of which should be carefully checked as the possible primary or contributory factor.

Vibration, instability of the fine-focus mechanism during exposure, or poor quality of objective are all possible. When achromatic objectives are employed, a possible cause can be introduced by previously focussing with a filter of one color (or no filter at all), followed by the introduction of a filter of a different color without refocussing.

In such cases of this kind, the answer can be found by a process of elimination. Any of these troubles, when finally located, can be eliminated in some manner, though not always without expense.

(2) *Faults due to improper exposures or development.* When this type of fault is present, it indicates a need for the beginner to become better educated in the strictly photographic technique involved. Outside advice from some friend who possesses experience in photography will be a big help here.

Pictures coming in this group are either flat, lacking contrast, even when the object was well stained and well differentiated, or they are

too contrasty and require a long time to print because the negative is extremely dense.

One can have a correct exposure, either under- or overdeveloped, or correct development of an under- or overexposed negative. In addition, four other combination conditions are possible: i.e., (a) underexposure, underdevelopment; (b) underexposure, overdevelopment; (c) overexposure, underdevelopment; and (d) overexposure, overdevelopment.

If one must work alone, without advice from someone qualified to give it, the place to begin to correct the trouble is with the development. By using the specific developer recommended by the manufacturer of the plates, at the correct temperature, for the corresponding time recommended for normal contrast, the development factor can be established. Such development of a test exposure, as outlined previously, should provide a standard for future guidance. There is, however, a simple visual test which can often be applied to a negative, that will indicate proper, over-, or underexposure, provided the development is substantially correct.

An underexposed plate held in such manner that a poorly illuminated part of the room is back of it, and with a bright light from a window falling on the top surface, will show a positive image with the film side up (i.e., toward the light and the viewer), but will not show such a positive image when the glass side is up.

An overexposed plate is just the reverse; a positive image is seen when the glass side is up, but the film side shows black. A properly exposed plate should show a very feeble positive image from both sides.

(3) *Faults due to an improper color filter.* The general tendency in the use of filters is to produce too much contrast. Parts of the object which ought to reveal considerable structure are portrayed absolutely black in the print. Often the photomicrographer himself is disappointed in the final results. The negative may look wonderful, with plenty of detail everywhere, but it seems impossible to make a print that looks like the negative. When the detail is in the shadows, the high lights are washed out; printing until the latter come out properly makes the darker portions black. The best that can be done under these circumstances is to use the softest available paper (#0 or #oo) and a very soft developer. In future cases, the place to correct the trouble is at the color filter. One should be chosen which does not make any portion of the object appear dark and lacking in detail. The proper contrast is that with which, using normal (#2)

paper, the very darkest detail in the object will just reach blackness in the development of the print when the whitest portion (possibly the clear background) is just starting to print. In this way the entire scale of the paper, from pure white to jet black is utilized.

There is one limiting condition, in this respect, for which there is no complete remedy. This occurs when an unusually dark object is to be photographed against a clear background. The filter must be chosen with reference to a proper portrayal of the object and the exposure must be ample fully to record the object on the plate. This means a gross overexposure of the clear background, which cannot be equalized, in all cases, by underdevelopment. The final result is a properly printed image of the object on a perfectly white background. This is by no means objectionable, however. As a matter of fact, some workers strive for a white background for every picture.

(4) *Faults due to improper illumination.* Here we may place blame for the majority of poor results in photomicrography. Failure to obtain critical lighting, improper centering of the illumination system, the introduction of diffraction effects, and other conditions of similar nature, all detract from ideal results.

When critical lighting is not employed, a very common result is an uneven illumination of the field. The result is a picture such as that shown in Figure 129. The variation in the intensity of the light, at the center and periphery, as seen on the ground glass, may not be evident visually, but the plate shows it. The cause of the dark border around an otherwise good picture is generally to be found in the vignetting effect of either the aperture or field diaphragm when they are not at the exact place in the system to perform their functions properly. With critical lighting — both diaphragms in their proper position — a vignetting effect is impossible. If the aperture diaphragm be closed down, it is manifested only as a darkening of the lighting over the entire area, but the lighting remains uniform. When the field diaphragm is closed, there should be no semishadow cast, but a sharp image of the edge of the diaphragm should circumscribe the diameter of the picture. When either of these diaphragms does not occupy its proper position, the vignetting effect results. The substage diaphragm, which is the aperture diaphragm, is far more susceptible to a slight change in position than is the field diaphragm; hence, as the substage condenser is removed from its proper position, carrying the diaphragm with it, the result may be an uneven illumination as shown in Figure 129.

Lack of centrality of the illumination system, combined with the

absence of critical illumination, is a very common fault, especially when vertical cameras are used. An improper setting of the mirror results in decentering the light. The resultant picture appears as in Fig-

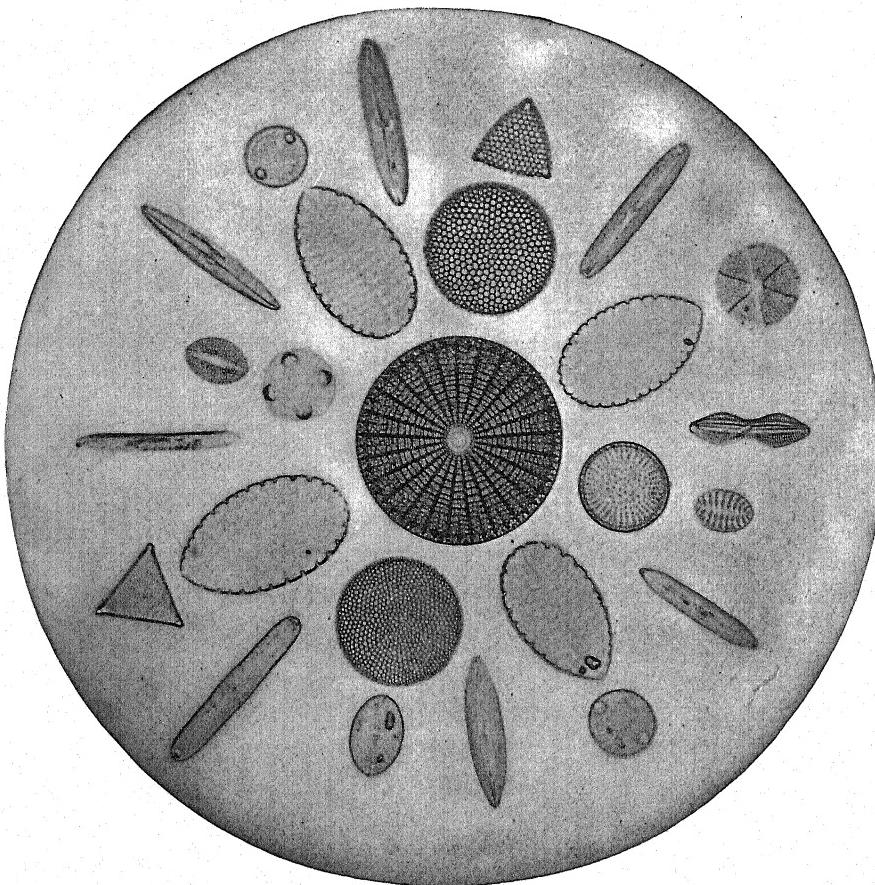


FIG. 129. UNEVEN ILLUMINATION

Caused by the failure to place condenser in the proper position for critical lighting, with a resultant vignetting effect produced by the diaphragms not being in the location where they can function properly.

ure 130. It is hard to detect on the ground glass visually. However, when the field diaphragm is sharply focussed on the plane of the object, as in critical lighting, this cannot occur, for the edge of the diaphragm is seen immediately any portion of it falls within the picture area.

Another common fault is the introduction of diffraction effects in photographing objects where diffraction is unnecessary to reveal the structure. Diffraction is necessary with unstained transparent objects, as illustrated by Figure 119, but when introduced in stained histologi-

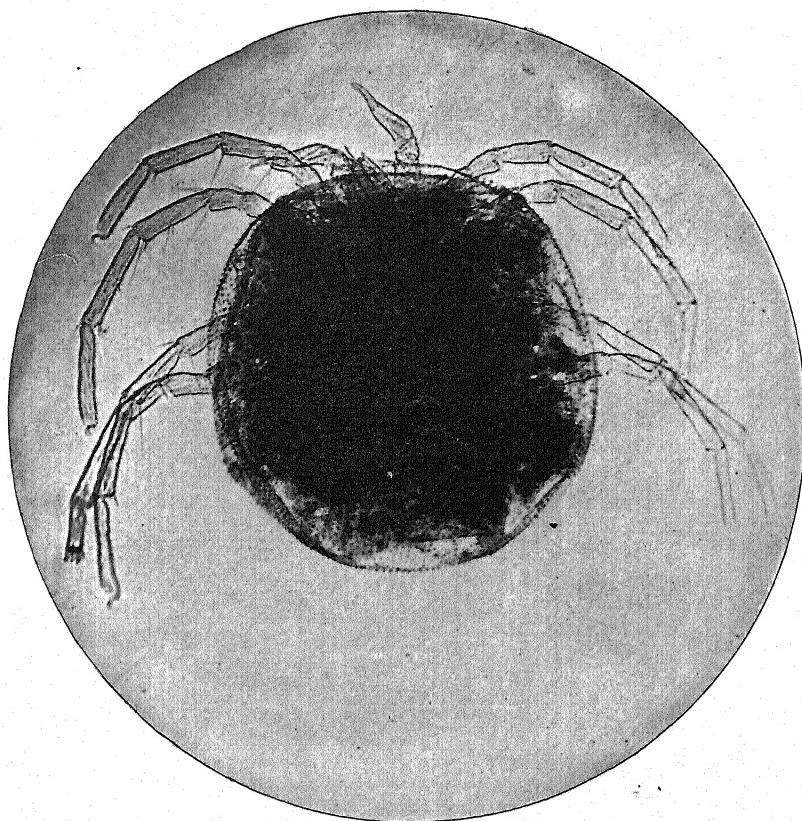


FIG. 130. LIGHTING NOT AXIAL

A condition caused by lack of critical illumination in combination with lack of alignment of the light with the optic axis. A common fault when a mirror must be used, as with a vertical camera.

cal sections, the effect is very unsatisfactory. The resulting appearance is shown in Figure 131.

Attempts to use objectives without eyepieces, or photomicrographic lenses in a small-tube microscope, usually produces a fogged area on

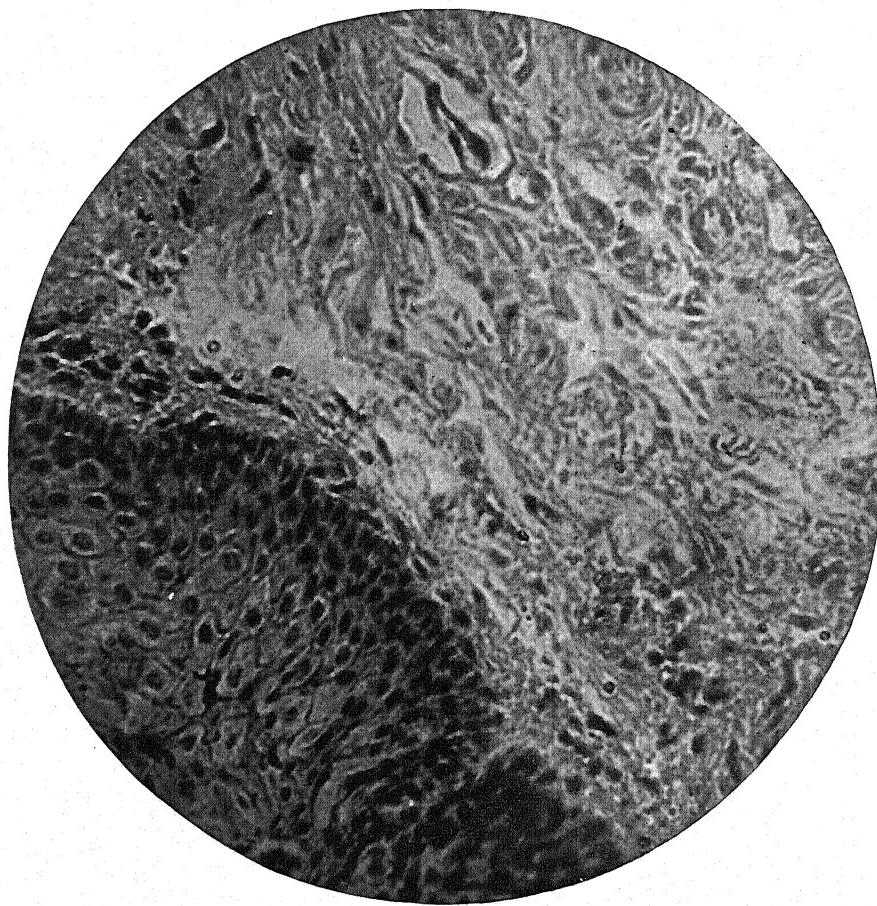


FIG. 131. EXCESSIVE DIFFRACTION CAUSED BY A NARROW CONE OF ILLUMINATION

All the darker elements in the tissue are seen to be surrounded by a white border. A correct focus is almost impossible under this condition, as that which appears to give the most striking image on the ground glass is not the proper one, as can be seen in this picture.

the picture, which results from internal reflections from the polished brass sleeve into which the eyepiece fits. The fogged spot may take the form of a solid white area in the center of the print or a light ring of larger dimension. Its size and shape are determined by the projection distance. The ring form is illustrated in Figure 132.

Insertion of a black paper tube or a metal sleeve containing an in-

ternal diaphragm of a diameter smaller than that of the eyepiece portion of the tube, will correct this trouble.

(5) *Faulty pictures due to improper magnification.* The particular faults in this group are not technical but, rather, aesthetic ones. The deviation from ideal results is largely confined to objects which are entities and should either be shown complete or only a small portion

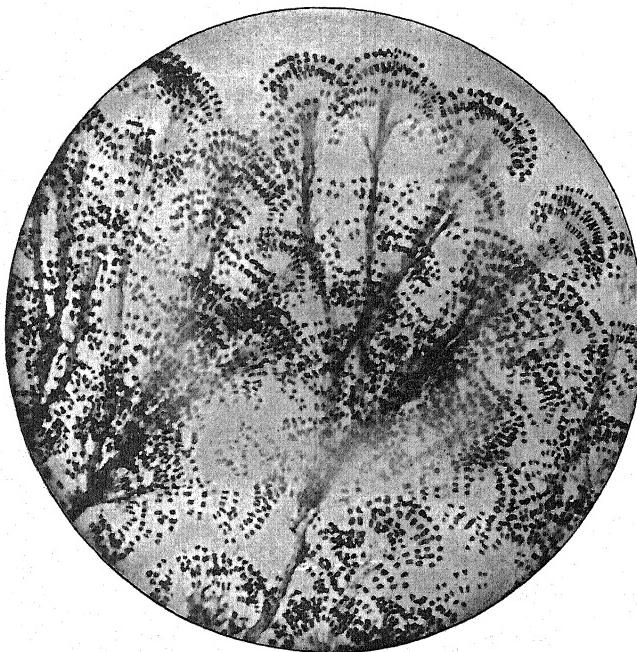


FIG. 132. FLARE RING FOGGING

A fogged circle, concentric with the image, produced by internal reflection from the microscope tube, when no eyepiece is employed and the light can project on the brass tube. Depending on the projection distance, the fogged area can either be a ring or a solid central spot.

at high power. For instance, it is an unsatisfactory picture which shows an amoeba entire, except for the end of one of the pseudopodia, which projects outside the picture. The condition is analogous to amateur snapshots, where the feet (or top of the head) of an individual are missing. From the artistic standpoint one is as bad as the other. A slight reduction in the magnification would have sufficed to include everything. Unfortunately, many pictures result from causes

beyond the control of the operator. The range of optical equipment and limitations in camera extension are responsible. Where these limitations are not present, however, one should strive to make his pictures aesthetically pleasing.

(6) *Unevenness of background traceable to plate emulsion.* This cannot be classed as a preventable fault, in photomicrography, since it is largely beyond the control of the operator. It is included in this list, however, as it may appear to be a defect in technique and cause many a headache in attempts to eliminate it.

This sort of unevenness of background is somewhat similar to the effect resulting from non-alignment of the light, although not so conspicuous. It will not be noticed on a negative, but, as a print is developed, the masked circle shows up at one side before there is any indication of printing on the opposite side. A combination of two conditions is required to produce the effect. The first is a slight difference in the thickness of the emulsion of the plate, which is very common though not necessarily universal. Superimposed on this unevenness of the emulsion must be a condition where the exposure of the background is so full as to affect the silver entirely through the emulsion. As previously pointed out, this must be done deliberately in many cases, in order that the object may receive ample exposure. As there is more silver in the thick portion of the emulsion, it builds up a blacker image and the final print is lighter on that side than on the side where there is less silver. Although this detracts from an ideal picture, it cannot be helped. It never occurs with sections and objects which fill the entire field.

Practical Advantages of Critical Illumination by Imaging the Light Source

In discussing the optical principles of photomicrography in Chapter 1, it was pointed out that critical illumination for medium- and high-power work can be secured by either imaging the light source or imaging the light condenser on the plane of the object. The latter method is employed in practically every complete commercial outfit sold in this country. The reason is obvious: it yields a uniformly illuminated field, regardless of the nature of the light source. For instance, a concentrate-filament tungsten lamp gives just as perfect lighting as the most uniform light source, whereas in the method of imaging the light source on the plane of the object one sees every coil

of the filament, superimposed on the object. Because of this, it would appear that there is nothing to be said in favor of the alternate method.

So far it has been assumed that the standard setup, using the light condenser as the light source, is employed. This has been done in order to present the practical aspects of photomicrography without complicating the problem in the mind of the beginner. Before leaving the subject of the technical aspects of photomicrography, however, there is something to be said in favor of the alternate method of obtaining critical illumination.

The standard method (often called the Köhler method), is, in spite of its beautiful uniform illumination, open to several objections. With a view to obviating these, the author experimented many years ago with a modified setup, utilizing the principle of imaging the light source instead of the light condenser. It has been so satisfactory that it has been used in fully 95 per cent of his work, for the past twenty years.

When a situation arises, as happens occasionally, where the so-called Köhler method will prove advantageous, a changeover can be effected within a few seconds.

A comparison of the advantages and limitations of the two methods may be helpful to many who have run into complications which they have not been successful in overcoming.

Experience has demonstrated that rapid exposures are not desirable for ordinary photomicrographic work. It is much simpler to work in the range of 1 to 60 seconds than in fractional-second exposures.

Further, an intense light is objectionable not only because it tends to fade many delicate stains and some gelatin filters, but because it is accompanied by excessive heat. Heat not only can damage a slide or an objective; it can also cause a change in focus due to expansion of various parts during exposure.

Both of these conditions are a factor when light from an intense source is imaged on the plane of the object either directly or as the condenser image. On the other hand, substitution of a lower-intensity light without further alteration in the setup does not give equivalent results photographically, as commercial low-intensity lamps are deficient in blue rays.

It is because of the heat effects that many commercial outfits include cooling cells, even with tungsten light illumination.

The light source in the arrangement ordinarily employed by the author is a 500-watt Ostram gas-filled tungsten lamp (rated $\frac{1}{2}$ watt per

candle). But this is not used as the effective light source, for directly in front of it are placed two finely frosted glass plates, the polished surfaces together. This provides a double diffusion and absolute uniformity over a circular area two inches in diameter. This disk of light constitutes the actual light source. It is located about one meter distant from the microscope. Directly in front of the frosted glass disks is located an iris diaphragm sufficiently large to utilize the entire two-inch circle of light, should it be necessary to do so. This diaphragm constitutes the field diaphragm. The field condenser is located on the optical bench so that the diaphragm lies in its principal focal plane, and thus the rays to the microscope are approximately parallel. They are intercepted by an auxiliary centering condenser of long focus (70 cm.) which converges them slightly into the substage condenser.

From the operating standpoint there is no difference in the procedure followed in effecting critical lighting, as described earlier in the chapter. After focussing the objective on an object, the condenser is focussed until an image of the field diaphragm is sharply defined on the plane of the object, the diaphragm being closed sufficiently to allow this to be done. The aperture of the substage condenser is determined in the usual manner by removing the eyepiece and observing the back lens of the objective.*

The majority of the illustrative micrographs in Chapter 8 were taken with this lighting arrangement. The question naturally arises, "What are its advantages over the so-called Köhler method?"

There are several. In the first place, no cooling cell is required. A simple test, made by placing the bulb of a chemical thermometer at the focal point of the substage condenser, with the diaphragm of the latter open, showed a rise of but one degree centigrade in five minutes. Removing the frosted glass and changing over to the Köhler method, with the same lamp, a rise of five degrees in three minutes occurred. This indicates that the heat is reduced to about one-eighth, a far greater reduction than can be obtained with a cooling cell.

In the second place, the exposure time is lengthened to a point where ideal exposures can be made and calculated exposures carried out to a small fractional percentage of error. That the frosted glass has little effect on the nature of the light, so far as altering filter values is con-

* The only commercial microscope lamp on the market that I am aware of which conforms to this design is the Ortho-Illuminator B, described in Chapter 2, page 000 and shown in Figure 57. I have been advised that a modified design of this illuminator adapting it for straight projection is contemplated.

cerned, is shown by the transmission curve of the two glasses (Figure 133). For comparison, a single piece of opal glass was also graphed on the same sheet. This shows that opal glass could be substituted for the frosted glass, both having nearly a straight-line transmission of between 40 per cent and 50 per cent. Either of these provides the one element ordinarily lacking to make critical lighting by imagining the light source, practical — i.e., a large area of absolutely uniform intensity over the entire surface. Though the exposure is practically

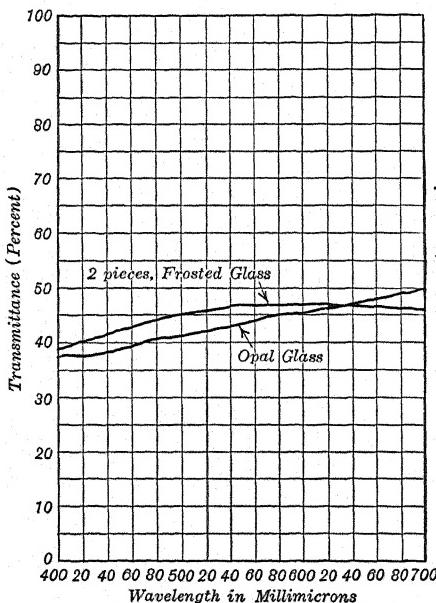


FIG. 133. Transmission Curves of Frosted and Opal Glass

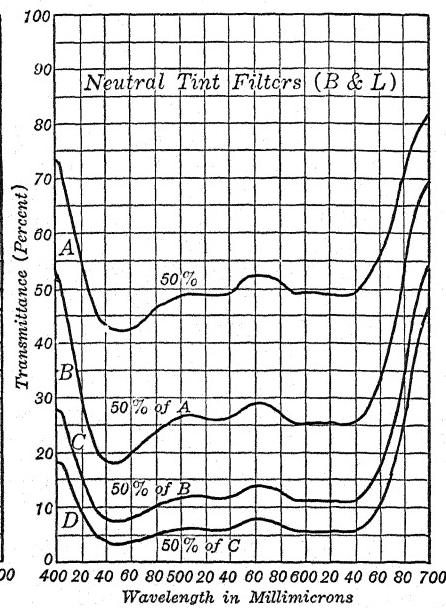


FIG. 134. Transmission Curves of B. & L. Set of Neutral Filters

doubled by the use of the glass, the heat is reduced many times more than the light intensity, giving an approximation of that ideal condition, "cold light."

Some workers feel that equivalent results can be obtained by the use of neutral-tint filters. The original set of filters supplied by Bausch & Lomb for this purpose, the transmission curves of which are shown in Figure 134 did not provide straight-line reductions in light intensity, and hence are not ideal for some types of work. These have now been discontinued and replaced by a set of four Inconel

metal deposited films with practically straight-line characteristics. Their transmission values are 50 per cent, 25 per cent, 12 per cent, and 6 per cent. While such filters are of value in visual work, the need in photomicrographic work for any of greater density than the 50 per cent transmittance filter is practically nil.

In this way maximum flexibility is secured. It should be noted that the heat problem is automatically taken care of, so far as the specimen and objective are concerned, by the use of filters of extremely low transmission characteristics. The cooling cell is still required, however, when an arc is used, as a protection for the filters which can easily be damaged by the extra heat which they would otherwise absorb.

Where an optical bench is not provided in the outfit it may be a little more of a problem to introduce the author's method of illumination, but wherever it is possible to use it, results will be found to be gratifying.

Special Photomicrographic Processes

Some types of photomicrographic work are of interest only to those engaged in specific or limited fields of research. While the general photomicrograph problems involved in these do not differ from those of ordinary lines of microscopy, in the majority of cases the work must be accomplished with specialized equipment, or at least with the help of auxiliary apparatus not required for ordinary purposes.

In order to avoid complicating matters for the general microscopist not concerned with these specialized techniques, they have been largely eliminated from consideration in Chapter 4. Some of these find very extensive application in their respective fields and the volume of work turned out entitles them to rank among the leaders in the photomicrographic field. Others are at present only of limited value, but advancing rapidly as their value to science and industry are being recognized. First on the list, in point of importance, can be placed the photomicrography of metals.

METALLOGRAPHY

Metallography, the science of studying, interpreting, and photographing the physical structure of metals by means of the microscope requires a radically different type of equipment from that employed for ordinary photomicrographic work. In the first place, the objects to be examined and photographed are entirely in the opaque class, and hence incident light alone must be employed. Although low-power work on metals can be accomplished satisfactorily by means of the various types of oblique illumination described in Chapter 4, by far the greater part of metallographic problems must be solved by recourse to high magnifications far beyond those possible with the methods available up until very recent years. The fundamental optics of metallography do not differ from those already discussed as applying to photomicrography as a whole. High-aperture objectives are re-

quired for high resolution; the illumination is subject to the same laws, as regards intensity in relation to magnification, etc., and hence a powerful light source, properly focussed on the surface of the specimen, is essential. Yet this must be accomplished even in the case of an objective which may have a working distance of but a few thousandths of an inch between its front lens and the metal surface.

The only solution lies in the employment of a vertical illuminator located above the objective, so designed as to receive a powerful light beam from the side, project it through the objective onto the specimen and allow the image picked up by the objective to pass it to form the image. There are several different methods of effecting this, so far as the design of the illuminator is concerned. The essential element in the illuminator is a reflecting surface which must lie in a 45°

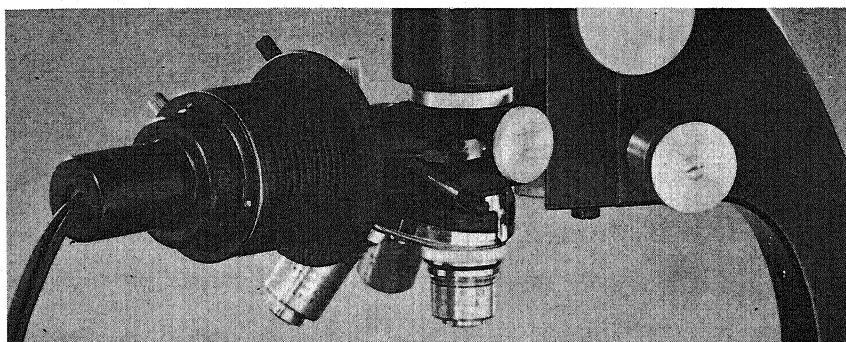


FIG. 135. Bausch and Lomb De Luxe Vertical Illuminator

position above the back lens of the objective. This reflector can be a 45° prism, located so that it just covers one-half of the area of the back lens of the objective, a semicircular mirror in the same position, double 45° prisms cemented together with half silvering on one prism, or a thin plain glass reflector. The latter is the commonly preferred form, for several reasons, among which are the reduced aperture, non-centralized lighting, and uneven illumination present in the prism illuminators. Figure 135 shows the Bausch & Lomb De Luxe vertical illuminator with lamp attached. This is an improvement on their earlier model in that it is provided with a triple nosepiece for quick change to other magnifications. Where more light is desired (e.g., for photomicrographic work) the illuminator can also be furnished without the attached light source, for use with an arc or other strong

light. This design is shown in Figure 136. For those not needing the triple nosepiece this illuminator can be supplied without it.

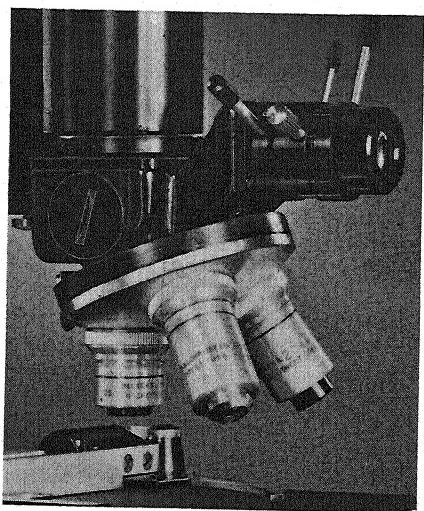


FIG. 136. Bausch & Lomb Vertical Illuminator Equipped for Use with an Exterior Light Source

micrography, especially at high powers, will require a stronger light source.

For occasional study of metals, simple metallographic stands such as are portrayed in Figures 137 and 144A suffice. A metallographic stand differs from a conventional biological microscope only in possessing a stage which can be raised and lowered. In this way, if need for photomicrographs arises it can be used in combination with a vertical camera, the beam of light being projected into the illuminator instead of against the mirror, as with transmitted light. It is because the position of the illuminator cannot vary with respect to the position of the light beam, that the stage adjustment is essential.

Instruments of this type can also be used in the horizontal position with large horizontal cameras. When this is done, the optical bench must lie at right angles to the camera axis. Where both metallographic and transmitted-light photography are to be done, two optical benches are required. The setup under this condition is shown diagrammatically in Figure 138.

For more pretentious metallographic work, where the interest lies

Bausch & Lomb also make a more elaborate type of vertical illuminator, the Tri-Vert, which by the use of special objectives provides for oblique as well as vertical illumination. This is shown in Figure 137, mounted on a CM stand. It has already been described in connection with similar equipment of other manufacturers. The principle on which it operates is illustrated diagrammatically in Figures 124 and 125 in Chapter 4 (see pages 204-5). Equipment of this sort is of special value in the examination of metals, especially of cracks, flaws, irregular surfaces, finishes, and the like; but as pointed out in Chapter 4, photo-

wholly in the realm of metals, the Le Châtelier design of microscope, often called the *inverted type*, is in general use. The surface of the metal to be examined is placed on the stage face down and it is illustrated in Figures 139, 140, Bausch & Lomb Company have largely pioneered in this design and offer three models to meet varying conditions as to requirements and price. The MILS Metallographic Equipment is an improved form of their previous ILS Metalloscope. This model metal to be examined is placed and 141.

The most elaborate Bausch & Lomb model is their Research Metallographic Equipment shown in Figure 142. It has a long bellows, taking up to 8" x 10" in picture size. The equipment is arranged to operate with either a zirconium arc lamp, a motor-driven carbon arc (unfortunately working only on direct current), or a low-voltage ribbon-filament lamp.

One important advance in this outfit is the employment of the Foster Calcite Vertical Illuminator.* The calcite prism in this illuminator is of special design and not only gives several times more light than a conventional glass-plate illuminator but yields a full cone of light. It provides plane-polarized light and in combination with a quarter-wave plate and glass cube can furnish either bright field or dark field illumination or completely polarized light equal to crossed nicols. It also possesses several other advantages.

Where price is a factor, a somewhat less expensive outfit is the Bausch & Lomb Balphot Metallograph, which takes a 5" x 7" picture instead of the 8" x 10" available on the more elaborate equipment. This does not have the new calcite illuminator. This metallograph is

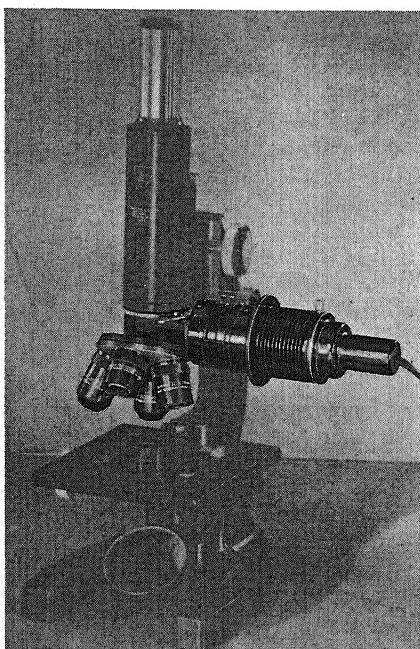


FIG. 137. Bausch & Lomb Tri-Vert Illuminator on a CM microscope

* Described in the *Journal of the Optical Society of America*, Vol. 28, April, 1938.

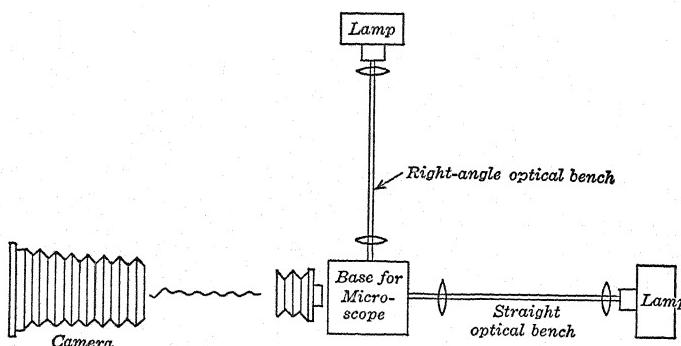


FIG. 138. Combined Straight and Right-Angle Optical Benches, Horizontal Type Camera

shown in Figure 143. One unusual feature of this instrument is the Magna-Viewer screen by means of which the field seen in the eyepiece can be viewed without recourse to the camera image. This viewer is shown in Figure 143, located directly in line with the eyepiece.

Other manufacturers, including the American Optical Company, Leitz, Zeiss, and Reichert, also supply research models of cameras for metallurgical work, employing the inverted stage design in the microscope. Each has its own modifications in design, that of Reichert being the most radical. It utilizes their Universal Camera Microscope MeF, equipped with a large horizontal camera and a zirconium arc. It is shown in Figure 144.

Metallographic photomicrography (although naturally of a some-

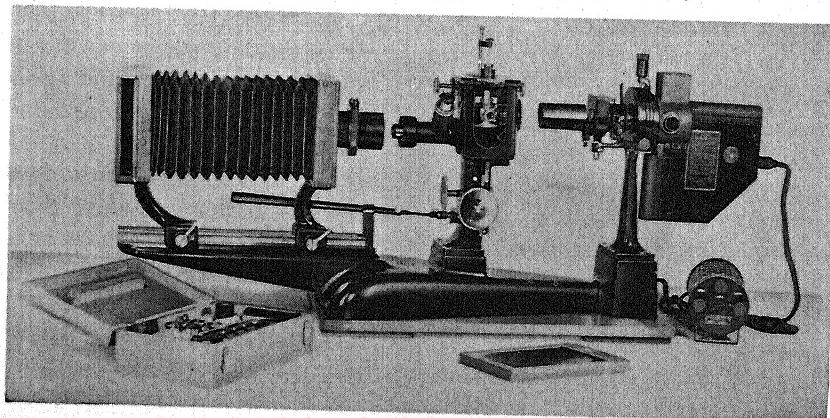


FIG. 139. Bausch & Lomb MILS Metallographic Equipment

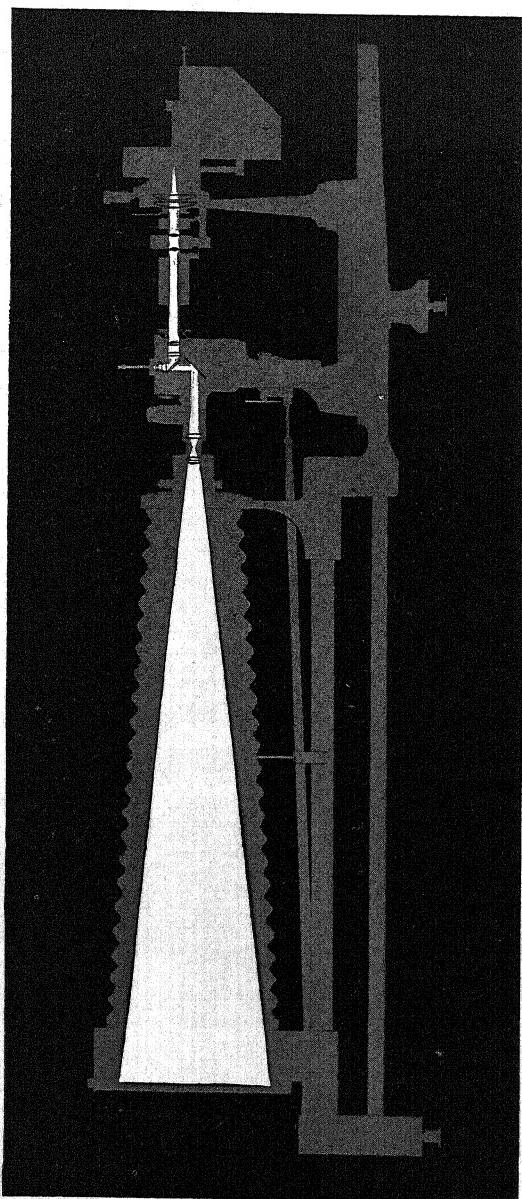


FIG. 140. The Path of Light Through Bausch & Lomb MILS Metallographic Equipment

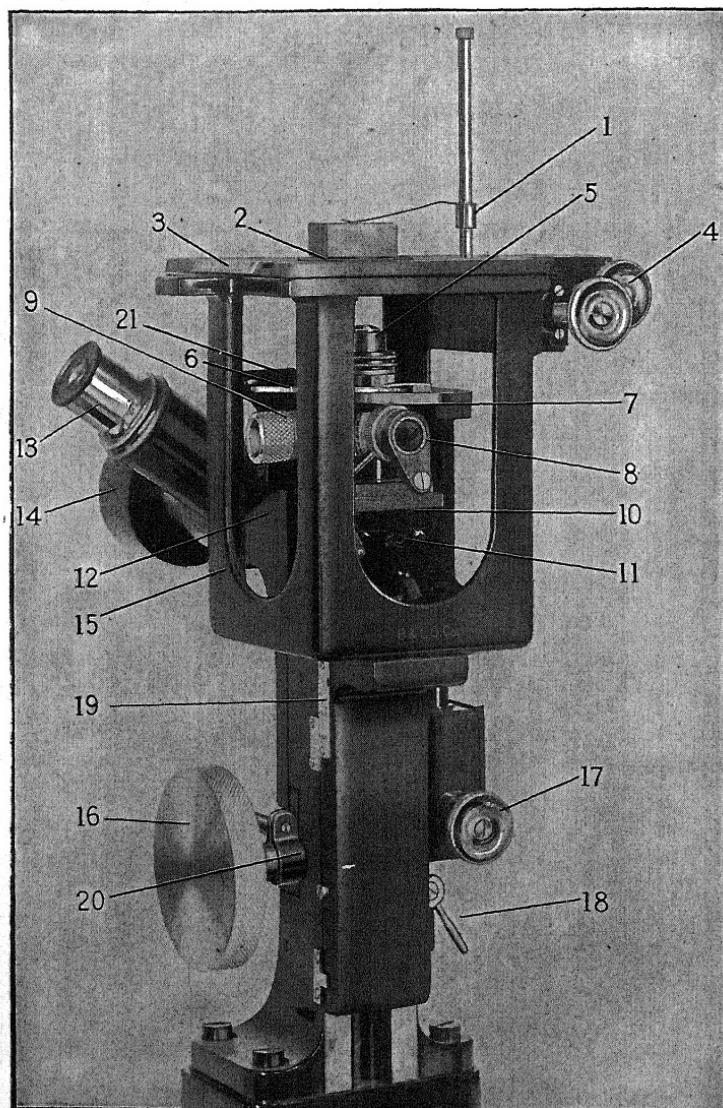


FIG. 141. MICROSCOPIC PORTION OF THE BAUSCH & LOMB MILLS OUTFIT

- 1. Specimen holder
- 2. Stage aperture plate
- 3. Mechanical stage scale
- 4. Mechanical stage adjustment heads
- 5. Objective
- 6. Objective handle
- 7. Iris diaphragm-adjusting ring
- 8. Filter mount
- 9. Vertical illuminator mirror mount
- 10. Stellite mirror housing
- 11. Heat-shield socket
- 12. Microscope body
- 13. Observation eyepiece
- 14. Camera connector
- 15. Stage casting
- 16. Coarse-adjustment head
- 17. Fine-adjustment head
- 18. Reducing gear lever
- 19. Coarse-adjustment scale
- 20. Coarse-adjustment lock
- 21. Stabilizer

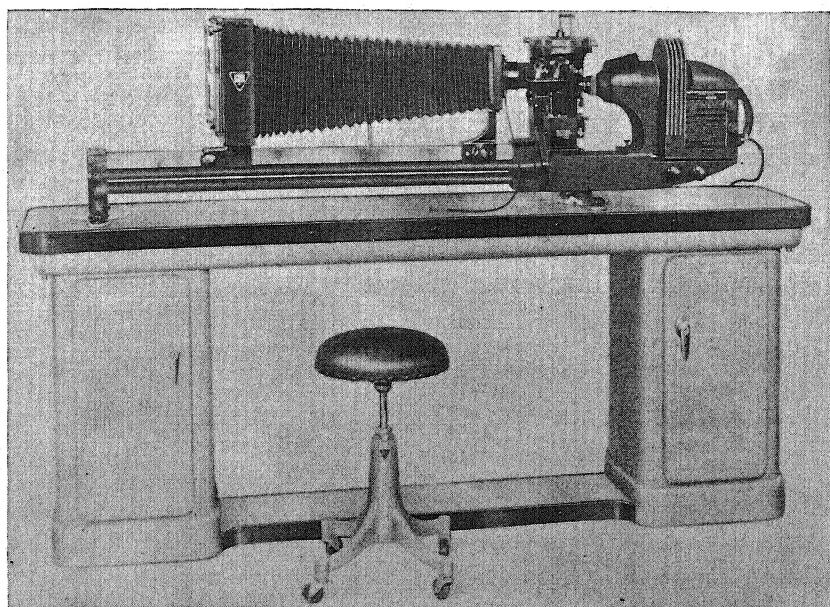


FIG. 142. Bausch & Lomb Research Metallograph

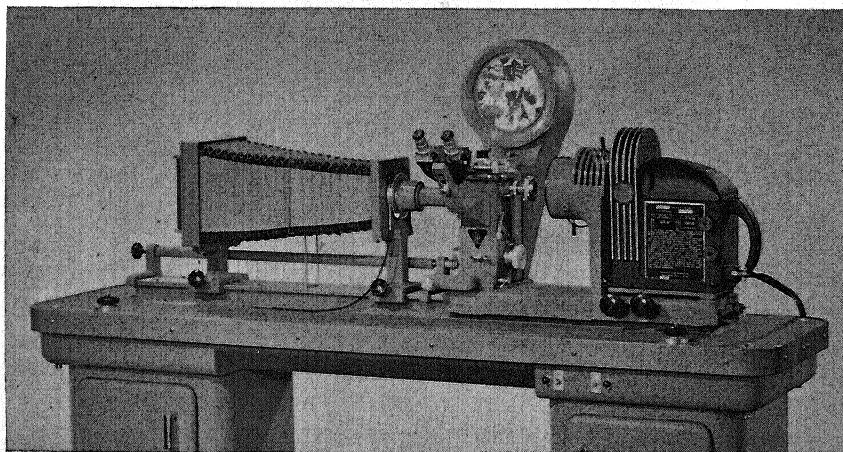


FIG. 143. Bausch & Lomb Balphot Metallograph

what limited kind) can also be done on the various self-contained outfits, as well as on many of the "universal" models, provided they are supplied with the equipment required for this type of work.

It is beyond the scope of this volume to enter into the technique of preparation of the metal samples, which includes polishing and etching. It is needless to add that no photomicrographs can be produced, no matter how perfect the technique of taking them, that are better than the samples themselves. It follows, therefore, that the highest quality of workmanship in the preparation of the samples must be assumed in dis-

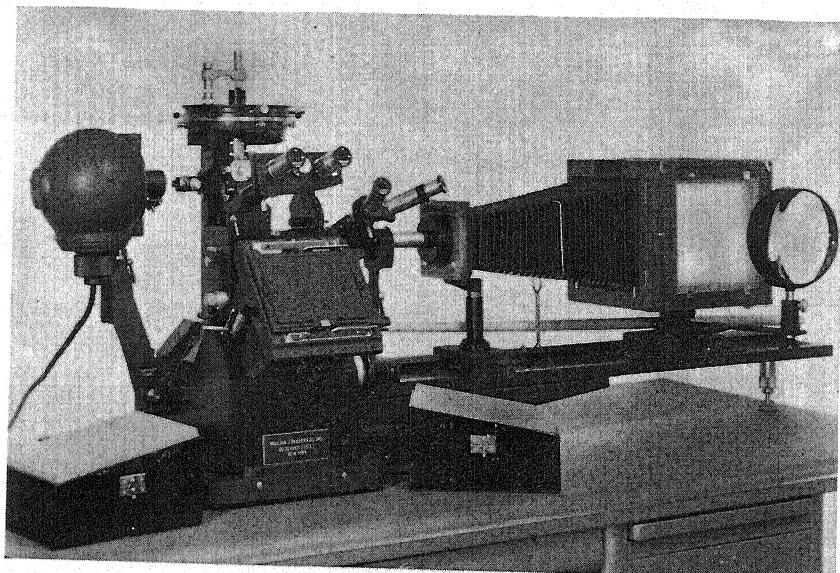


FIG. 144. The Reichert Metallographic Outfit

cussing the strictly photomicrographic aspects involved. For anyone interested in taking up this line of work, whose sole previous experience has been along general microscopical lines, it may be well to point out a few places where considerable divergence occurs in the optics of the two conditions.

Metals differ from ordinary microscopical objects in that no cover glass is employed. As the cover glass is a part of the optical system of the objective, omission of the cover glass makes it obligatory to employ an entirely different series of objectives, especially designed to work without cover glasses. This does not apply to homogeneous immer-

sion lenses which will work equally well in either case, so far as presence or omission of the cover glass is concerned, but even here other conditions enter to make a distinction between the lenses required. These are: first, the desirability of having the lenses of the objective located as close as possible to the vertical illuminator, in metallographic work, in order to reduce reflections; and second, the employment of a longer tube length in metallography.

The appearance of a metal specimen is entirely different under ver-

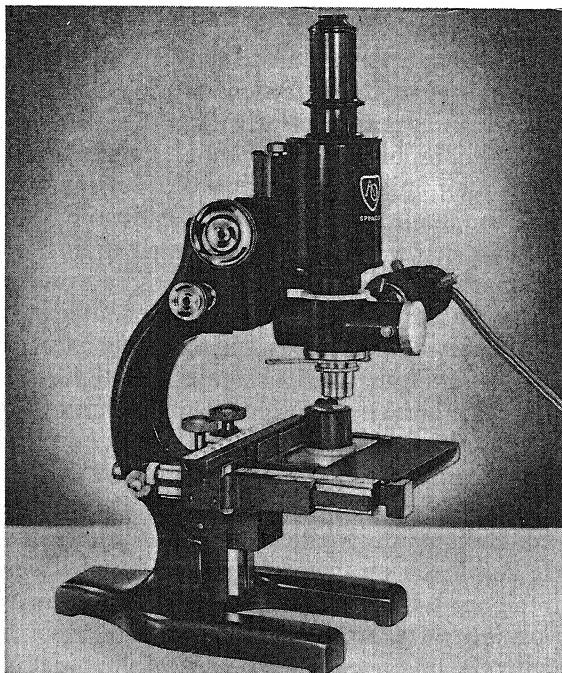


FIG. 144A. American Optical Company Microscope with Graduated Mechanical stage.

tical illumination from that under oblique lighting. The reason for this may be understood if we compare what happens in the case of a highly polished surface when illuminated by the two methods. Such a surface behaves as a mirror, so that if we are observing it under a microscope with oblique light falling upon it, according to the law that the angle of incidence is equal to the angle of reflection, the total light would be reflected on the side of the microscope opposite to that from which it is illuminated. No light would be reflected *into* the micro-

scope, and the specimen would appear dark. But with vertical illumination, all light is reflected directly back into the microscope and a dazzling white surface results. In other words, the two appearances are exactly opposite. It is for this reason that vertical illumination, which must be employed with high powers, is adopted for low-power work also. Interpretation of micrographs can thus follow a consistent system, regardless of magnification. When for some reason it is found desirable to utilize a different type of illumination for a polished and etched metal specimen, in taking a photomicrograph, the method should always be stated, as otherwise even an expert metallurgist might not be sure as to whether the effect was obtained by illumination or by some unusual etch, since varying the etchant can also result in similar reversals in appearance. Although the fundamental optics applying to metallographic work are not essentially different from those of general photomicrography, it is not so apparent, with unit outfits such as the Bausch & Lomb outfit, just what is involved, for everything has been so designed that proper optical conditions are met. One has only to follow the operating instructions furnished with the outfit. Nevertheless, one is aided to proper understanding of the equipment by becoming familiar with the theoretical principles of illumination underlying the practical setup.

When working with the conventional form of microscope equipped with a vertical illuminator, the need for a thorough knowledge of the principles of illumination is greater, for in this case the entire arrangement of the light train is left to the ingenuity of the individual.

It is preferable in metallographic work to utilize the method of imaging the light condenser on the plane of the object. This means that the light source must be focussed on the rear focal plane of the objective. This position varies somewhat with different objectives, but is approximately at the opening into the vertical illuminator, or the diaphragm of the latter when it is equipped with one. When the light is imaged on the back focal plane of the objective, the rays pass from the objective to the metal surface substantially parallel, illuminating the maximum area which can be covered by the objective. My preferred method of accomplishing this illumination is to have a parallel beam from the lamp condenser, located at the distal end of the optical bench, enter a 20-cm. (focal length) condenser which projects the beam into the vertical illuminator. This condenser can be moved on the optical bench to adapt it to each objective, but the usual distance is around its focal length from the diaphragm of the illuminator. The

latter serves as the aperture diaphragm, while a second large diaphragm is located between the illuminator and condenser (about 6 or 7 cm. from the latter). This serves as a field diaphragm, and being so near the microscope, is under control at all times.

While exact critical illumination is not so imperative in the case of metallographic work as with transmitted light, the nearer it is approximated, the better will be the results. Care must be taken not to allow a larger cone of light to be projected into the illuminator than can be accommodated by the back lens of the objective, for it will only cause annoying internal reflections. With this system of illumination it is possible to block out the central portion of the beam by means of a disk in the center of the aperture diaphragm. This is called conical illumination. It tends to introduce some shadow effects, especially when the disk is decentered slightly, or when a segment of the outside light cone is also suppressed.

If achromatic objectives are to be used for metallographic work, superior results will be obtained by the use of a green filter, since this is the particular spectral band for which the objectives are corrected. As arc lamps are used almost exclusively for metallographic work at high magnification on account of the low efficiency of vertical illuminators of the plain glass type (as much as 90 per cent of the light may be lost), a high amount of ultra-violet is present. As plates are usually quite sensitive to short waves and as achromatic objectives are not well corrected in this region, it is desirable to eliminate all the shorter waves, or an inferior image will result. Plates sensitive to green must be used in this case. Kodak Metallographic plates are especially suited.

Wherever possible, apochromatic objectives should be employed, as blue filters can be employed with these and the resolution improved materially. Color-sensitized plates are not required with blue light.

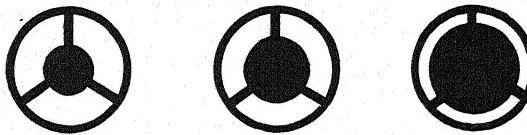


FIG. 145. Diaphragm Stops for Dark Field

DARK FIELD PHOTOMICROGRAPHY

Dark field microscopy plays a very large and important role in certain specific lines of research, notably bacteriology and colloidal chem-

istry. Its value in revealing extremely minute particles in suspension and particularly living, unstained bacteria in a hanging-drop culture, is largely responsible for its frequent application in such lines. On the other hand, the photomicrographic aspect of dark field is of lesser importance. This is due in part to the difficulties involved in taking the extremely short exposures required for moving objects. A second reason is that little structural detail is evident in the average dark field micrograph; further, the diagnostic value of the moving particles or organisms present in visual dark field work is largely lost in a still picture. For instruction purposes, however, a definite need for dark field micrographs exists and a photomicrographer should be prepared to take such pictures when they are required.

From the strictly scientific standpoint, dark field microscopy is confined exclusively to high-power work. The amateur microscopist, on the other hand, delights in the beautiful appearance of larger organisms when illuminated brilliantly on a dark field. Low-power dark field condensers, although available commercially, are not in common use, nor are they necessary, because a central stop, such as those shown in Figure 145, interposed underneath an ordinary substage condenser is as effective as a specially designed condenser would be. There are several forms of high-power dark field condensers available. Their principle of operation is essentially the same as that employed for low-power work with a central stop in combination with an ordinary condenser. The two most commonly used for photomicrographic work are the Paraboloid and the Cardioid (or bispherical). The former provides a wider latitude in operation, as the focal point is not so critically defined, while the latter gives a more intense illumination, providing the condenser is accurately focussed on the plane of the object. The path of the rays in the Cardioid condenser is illustrated in Figure 146.

The problems involved in dark field photomicrography are determined largely by the nature of the object. Where living organisms in motion are involved, or Brownian movement (*pedesis*) in colloidal particles is present, a fractional-second exposure is necessary, the exact time allowable depending on the speed of movement. As magnification amplifies the apparent speed, as well as the size of the object, keeping the magnification as low as possible is a help, since the intensity of the light varies inversely as the square of the magnification. This means that to reduce the magnification to one-half, the exposure is only one-quarter as long and the movement reduced proportion-

ately. Fast plates are also desirable for this class of work. Enlarging the negative to the original size (i.e., two times) gives the desired object size with only one-quarter the motion.

Where motion is not present, and minute organisms must be shown as contrasty as possible, both the exposure and development of the negative should be on the long or contrasty side. On the other hand, when a decided structure is present and can be demonstrated, the exposure should be sufficiently long to register detail in the darker portions, while the development must be on the soft, or less contrasty, side. It is better to use a contrast developer in the first case and a soft developer in the second. Sometimes it pays to change plates as well.

Every attempt should be made to keep the field non-illuminated so it will appear black in the finished micrograph. It goes without saying that only an arc lamp will give ideal results in high-power work. Low-power dark field micrographs, however, can be successfully made with a 500-watt tungsten lamp.

Unless one is so experienced that he can tell the order of magnitude of the exposure necessary under varying conditions, it is worth while to make a test strip exposure, at the start of this type of work.

Photomicrography with the slit ultramicroscope is in the same class as dark field work and subject to the same conditions in the photography of colloidal solutions. Very little of this type of work is called for, or justified. When it is necessary for some special reason, the use of the most rapid plate or film (supersensitized) and the least possible projection distance will give satisfactory results.

The photographing of colloidal solids under the slit ultramicroscope can be accomplished without difficulty, as the exposure can be prolonged to the desired extent. For this reason, slower and more contrasty plates can be employed.

Another type of illumination which can be classed with dark field,

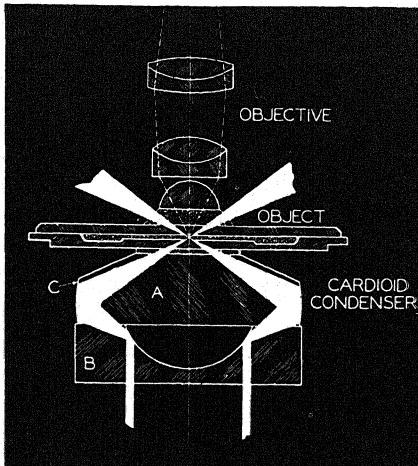


FIG. 146. Path of Rays in the Cardioid Dark Field Condenser

because it is based on the same principle, is that known as Rheinberg illumination. This form of lighting employs two differently colored filters to produce the desired effect. One of these (possibly of a blue color) is a disk just large enough to fill the full aperture of the objective. This is set in the center of another color (red, for example) of a contrasting nature, the outside disk being the full size of the substage condenser. Such a composite disk is illustrated in Figure 147. When such a disk is placed under the substage (the diaphragm being wide open), the object appears brilliantly illuminated, the color of the outer ring, while the field has the color of the central disk.

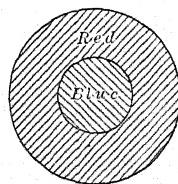


FIG. 147. Rheinberg Disk

There is not much advantage in photographing in black and white with these disks, although if a considerable series of them is available, in various color combinations, some pleasing effects can be secured for certain objects. In deciding what combination is to be used, one must merely consider that two filters are being employed, and judge the effect on the basis of what each filter will accomplish alone, when used with the particular plate being employed. The ideal use of Rheinberg illumination is to be found in connection with photography in natural colors, which we shall discuss later.

PHOTOMICROGRAPHY WITH POLARIZED LIGHT

Occasions arise in many types of microscopical work where illumination by means of polarized light is required. Chemical crystals, petrographic sections, starches, fibers, and various animal and vegetable tissues are included among the types of objects which can be studied to advantage by means of polarized light. All of these, at times, must be photographed as they appear under this type of illumination.

Although instances occur when surfaces of opaque objects must be examined, and possibly photographed, by means of polarized light, by far the greater portion of work with this form of illumination comes in the transmitted-light class. It should, therefore, be treated just like any other transmitted-light work so far as photographic problems are concerned. The use of critical lighting is desirable for the best results, and the effects of magnification, numerical aperture, and other factors are identical.

There are, however, problems in photomicrography with polarized light peculiar to this type of work. These relate to the presence of

astigmatism when a prism is used as the analyzer; covering a large area in low-power work; correct rendition of interference colors in black and white; presence of extinction in some portions of a section when the polarizers are crossed; photography of interference figures; and others of like nature.

When a modern high-quality petrographic stand is employed, some of these problems are automatically solved. For instance, astigmatism, caused by the presence of the nonsymmetrical prism in the path of the image-forming rays, is compensated by means of a cylindrically ground lens associated with the prism. Astigmatism is not so visually noticeable since one is subconsciously manipulating the fine adjustment and to some extent compensating for it. The effect of astigmatism is to make lines in the object which lie in one direction, out of focus when those at right angles are sharp. Altering the focus reverses the condition.

In a photomicrograph when astigmatism is present, either set of lines can be sharpened, at the expense of the other, but a compromise focus is sometimes preferable.

Due to the high cost of large prisms, and the scarcity of suitable ice-land spar with which to make them, it is extremely difficult to secure prisms sufficiently large to cover low-power fields and the full aperture of microphotographic lenses. This problem has been solved for many classes of work, in recent years, with the development of Polaroid and other polarizing materials of a similar nature. Polaroid can be substituted for both the polarizer and analyzer. Astigmatism is not present when it is used, but other conditions are introduced which must be reckoned with.

Polaroid acts by the partial absorption of the rays vibrating in one direction. When the films are crossed, i.e., in what would be the position of extinction, there is considerable residual light in the red remaining (and occasionally in the blue), and hence the effect is not one of complete blackness, but a decided red or purple-red color persists. Figure 148 shows the spectrophotometric graph of Polaroid, double in parallel position, and crossed.

Except for slight surface reflection and transmission losses, prisms of any type made from calcite will show a substantially straight line 50 per cent loss for one prism and zero transmission when crossed. These differences must be taken into account when photographing with Polaroid.

Nicol prisms of the conventional type with slanting faces are quite

unsatisfactory as analyzers, for photomicrographic work. The flat face modified form of Nicol prism, Ahrens or Abbe prisms are far superior. The Abbe triple-prism type does not bend the rejected ray at a very great angle, but it is usually sufficiently far removed so as not to strike the plate.

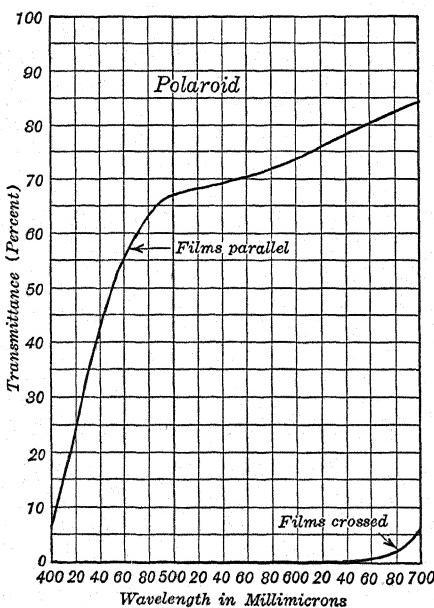


FIG. 148. Spectrophotometric Curve of Polaroid Films

proper differentiation of the colors in the image will be obtained in the picture. The reason for this is obvious. Two mineral grains may lie side by side, one a bright green and the other a rich red, visually, in the position chosen for the picture. Yet if the total transmission of light in each happens to be of the same order, they will photograph alike and not even the boundary between them may show. An eye trained to appreciate variations in light intensity, apart from color, is a great asset in this work, for it usually is possible to find a rotational position where maximum differentiation on the basis of intensity will provide the best picture.

Photography of polarized-light effects in natural color is the ideal solution, wherever possible.

In recent years much has been done with the application of polarized light to the study of metals, where the nature of reflected

One of the most serious complications encountered in the photographing of rock sections is the difficulty of eliminating the condition of a considerable percentage of minerals lying in the approximate position of extinction. No matter how a section may be turned, as one grain becomes light, another darkens. It is often possible to ameliorate this situation either by turning the analyzer slightly out of the 90° position, or by inserting a selenate plate in the system, between the polarizers.

Panchromatic plates should be used for polarized-light work, but even with these there is no assurance that a

light produces the interference effects. Since polarization occurs through reflection from surfaces at critical angles, etchants on metal surfaces which develop crystal surfaces on individual grains in a metal will yield polarization interference colors. Thus the nature of the crystal grain orientation can be made evident. Light projected on the metal surface must be plane-polarized and the reflected beam must be analyzed by crossed polarization. The Bausch & Lomb Research Metallographic Equipment, by means of its calcite illuminator, can accomplish this. With other types of metallographic stands, a polarizer must be inserted in the light beam entering the vertical illuminator and an analyzer placed above the objective. Metal photography in polarized light must be done in color to derive any benefit from it in picture form.

Photographing interference figures in crystals is simple when a microscope equipped with a Bertrand lens is available. Where an ordinary microscope is employed and the analyzer is located directly above the objective, a Bertrand lens can often be improvised by mounting a low-power objective (about 2" focus) in the threads at the bottom of the draw-tube. The latter is then used to obtain the correct focus by sliding it in and out. Conoscopic observation of interference figures requires the use of at least a 4-mm. (40 \times) objective and a full cone of illumination from the condenser. Any arrangement which will provide a visual interference figure will suffice for photographing it, but it must be understood that it is the Bertrand lens and *not* the objective which is to be focussed to obtain a sharp image.

PHOTOMICROGRAPHY IN NARROW AND MONOCHROMATIC SPECTRAL REGIONS

Photography in narrow spectral regions is but a specialized modification of ordinary color filter work. It differs, so far as the region of the visible spectrum is concerned, largely in the nature of the light sources employed.

As a matter of fact, only two of these are of interest to the photomicrographer. Both are, to all practical purposes, strictly monochromatic. This monochromatic characteristic is quite different from the narrowest band possible with filters, since but one specific wave length is utilized.

For one of these an electric sodium lamp is employed. In a lamp of this type (Figure 59) a little sodium metal is vaporized by means of a heating element until the electric current will arc across two terminals through the sodium vapor, which is made to emit its typical spectrum. The spectrum of sodium consists of very few lines in the visible region, all of which are of relatively small intensity, except that known as the D line, which is an intense yellow.*

When a sodium lamp is used as the source of illumination no filter is required, since only light of substantially a single wavelength is emitted. Photography by means of the yellow D line is of value under two different conditions. One is that chromatically, at least, the performance of the objective is perfect, even though the lens itself is far from ideal in its correction for chromatic aberration. There can be no chromatic aberration when there is but one wavelength of light involved. If, therefore, the spherical correction in this region is good, a fine image results.

But a still more important advantage may lie in the relative absorption by different elements in an object, of a specific wavelength; hence a radical differentiation may be achieved in a photograph, such as is scarcely, or not at all, evident to the eye, and which will vanish completely, even on a photographic image, when a slightly wider spectral band is employed. This stage of photomicrographic research is still in its infancy and may result in many new discoveries when it is more fully employed. (See Plate XXIX in Chapter 10 as an example of this.)

The other wavelength in the visible spectrum which is available and frequently employed is a powerful green line present in a mercury arc lamp. The mercury lamp is somewhat similar in principle to the electric sodium lamp, but no preliminary heating is required, since mercury is a liquid at ordinary temperatures and can establish its own circuit to form the required arc. There is one essential difference between the mercury and the sodium lamps in their application to photomicrography. While mercury only emits a few wave lengths in the visible region, there is not the same difference in their relative intensity and they are more widely scattered. Moreover, there is a very power-

* The D line is one of the series of prominent black (absorption) lines seen in the solar spectrum. Actually it is double, but the two lines are so close together (5889.97 and 5895.93 angstrom units) they usually appear as one in small spectrometers. Two other pairs of lines are also present, one on each side of the D line (5683-5688, and 6154-6161) but they are not sufficiently strong materially to affect the plate during the time required to make an exposure with the D line.

ful line in the ultra-violet which has more effect on a photographic emulsion than any other. For this reason a filter must be used in combination with a mercury lamp to eliminate all but the desired line. The Kodak Wratten filter #77 cuts out practically all but the green line at 5460 a.u. This line is passed at about 70 per cent efficiency, so that we have available another monochromatic light source, in addition to the Sodium D line.

It would be equally possible to isolate the 4358 a.u. (violet) line in the mercury arc, by means of a suitable filter, but the relative insensitivity of the eye to the blue-violet region makes focussing difficult, or even impossible. Since this is the case, there is little advantage in using this line, because, by an ingenious method, which will be discussed later, it is possible to utilize the strong line lying completely within the ultra-violet region, at 3650-3655 a.u. (double). Either panchromatic or orthochromatic plates sensitive to green and yellow must be employed with both the sodium and mercury (green) lines.

There are two narrow band regions where photomicrography can be carried on to advantage with ordinary equipment. These are the near ultra-violet and the infra-red.

Both Kodak Wratten filters mounted in glass and Jena glass filters are available for these regions. For the ultra-violet end, though mercury vapor lamps can be used, arc lamps will provide a more intense source; if direct current is available, the most powerful source of all lies in the iron arc, operated at around five amperes.

Ordinary glass lenses will transmit light between 3000 and 4000 a.u. although the balsam used for cementing the components will fluoresce a little. Microscope objectives are not so well corrected in this region and it can be expected that the focus will not be the same as for the shortest blue-violet rays which the eye can use (which, of course, are longer than 4000 a.u.). One cannot be sure, until a test is made, just how good an image can be produced with his particular lenses, but it is worth trying, whenever an occasion arises where a picture in the ultra-violet region is desirable.

There are two possible methods of determining the focus. The preferable, and by far the quicker one, when the illumination is sufficiently intense, is by means of a strongly fluorescing glass plate of uranium glass. One of the best for the purpose is the Jena glass filter GG-12. A glass of this type, mounted on the plane of the sensitized plate (i.e., in lieu of the ground glass, which must be removed) and

viewed by means of focussing glass (Figure 82), will reveal the image, clearly fluorescing on the glass. Where this cannot be done it is necessary to make a set of test exposures, preferably by means of a multiplicator back, to determine the proper position. The procedure is as follows:

First, secure the best approximation of the focus by means of the blue-violet (C) filter; replace the latter with the ultra-violet filter and run a series of test exposures to determine the proper exposure time. Having found the latter, note the position of the graduations on the fine adjustment knob, rotate it a few microns so as to back the objective *away* from the object, and record the position. A series of test strip exposures should now be taken on a multiplicator, starting with the recorded position of the graduations on the fine adjustment, the knob being turned so as to bring the objective one micron *toward* the object in each subsequent strip. The total number of strips should be such that the position of best visible focus with the blue-violet filter will lie somewhere near the central exposure. This strip test will indicate in which direction from the visible position, if there be a divergence, the correct ultra-violet focus lies. It may, by chance, show the proper focus. Should one of the end exposures appear the best of the series, a second test plate should be made to extend the exposures past the correct focus. One may also judge, by the difference between the individual exposures of the first test, whether a larger spacing or a still finer step should be used. In general, low-power objectives are not so sensitive to slight variations in focus, while high-power oil-immersion lenses require intervals of the order of one-half micron. When once the variation between the visual blue-violet and ultra-violet focus has been determined for a given lens, it should be recorded in the photomicrographic notebook. Subsequent micrographs can then be taken by the simple expedient of focussing with the C filter, moving the graduations on the fine adjustment knob in the proper direction the amount of correction necessary; and then changing filters and exposing.

When Zeiss apochromatic objectives are available it is not necessary to follow this procedure, since this firm supplies auxiliary sleeve lenses to slip inside of the regular series to correct them for use in the ultra-violet region. They also make a similar correcting lens for use in the infra-red.

Ultra-violet work does not require color-corrected plates; the slower, finer-grain plates of the process and near-process class are

superior and equally fast in the short-wave region. It is not to be expected that the type of ultra-violet work described will be used especially for increasing resolution, since the characteristics of the objectives, other than the chromatic focus, are not altered by the correction in focal position. The special advantage in this type of ultra-violet work lies in the demonstration of, and utilization of, variations in the absorption of ultra-violet light in the region of 3000 to 4000 a.u. This, of course, is band absorption, as distinguished from absorption at one definite wave length.

Just as it is possible to work in the short-wave region lying beyond the visible range, so can we proceed with the non-visible rays at the opposite end, the infra-red rays.

Infra-red filters covering different bands in the infra-red region are available in both the Wratten and Jena glass series; these can therefore be chosen so as to utilize the particular region desired. The procedure as to establishing the correct focus for the objective is identical with that described for ultra-violet photography except in the substituting of the F filter for the C in the visual part of the operation.

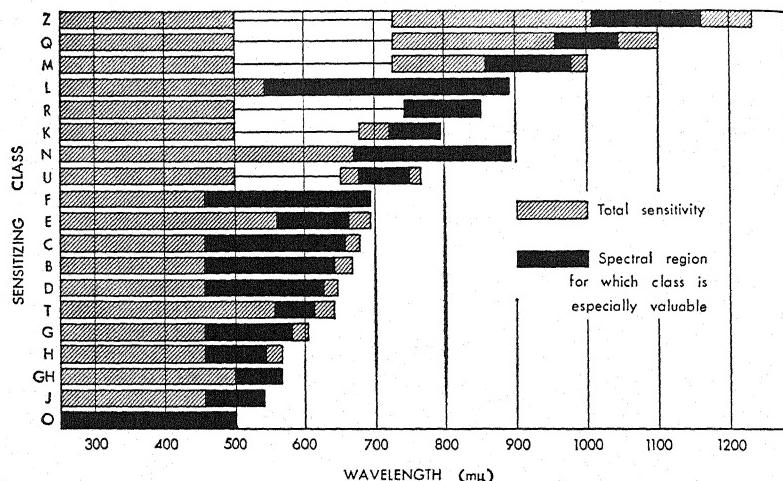
An arc lamp should not be used for infra-red work, since it is relatively poor in the longer waves as compared to a tungsten lamp. To obtain an equivalent effect, there is much excess visible and ultra-violet illumination to absorb. A tungsten lamp, working at reduced voltage, is superior. It is desirable to use a light which provides enough visible red to accomplish the focussing; otherwise a strictly infra-red glow lamp would be ideal.

Infra-red photography requires photographic plates corrected for use in this region. Such plates must be ordered specially from the factory, since they are not a stock item. Those interested in this work should secure from a Kodak dealer copies of, *Kodak Photographic Plates for Scientific and Technical Use*, and *Kodak Materials for Spectrum Analysis*, both of which are sold at a nominal charge. The accompanying chart of plate sensitivities is taken from these booklets. For most photomicrographic work in the infra-red the 1-N plate shown on this chart is ideal and within the range where ordinary apochromatic objectives may be expected to function satisfactorily.

Far more microscopical research work in both the ultra-violet and infra-red bands, as well as in narrow bands in the visible spectrum, should be done than has been attempted to date.

To this end, recent developments in filters provide means for investigations in narrow spectral bands in all regions of the spectrum

SPECTRAL CHARACTERISTICS OF KODAK SPECTROSCOPIC PLATES^a



Emulsion and Sensitizing Combinations Available:

Sensitizing Class	Emulsion Types									
	103a	103	I	II	IIa	III	IV	V	548	649
O	103a	103	I	II	IIa	III	IV	V	548	649
J	103a	103	I	II	IIa	III	IV	V
GH	V	*	649
H	103a	103	I	II	III	IV	V
G	103a	103	I	II	III	IV	V	548
T	103a	103	I	II	III	IV	V
D	103a	103	I	II	III	IV	V
B	103a	103	I	II	III	IV	V
C	103a	103	I	II	III	IV	V	548
E	103a	103	I	II	III	IV	V
F	103a	103	I	II	III	IV	V	548
U	103	II	III	IV	V
N	I	IV	V
K	II	III	IV	V
R	II	III	IV
M	I	IV
Q	I	IV
Z	I	IV

*Supplied as Kodak High Resolution Plate

^a By permission of Eastman Kodak Company.

within the range of 400 to 1000 millimicrons, with transmission up to 45 per cent.[†] While these filters are of especial value in research

[†] The Schott Narrow-band Interference Filters are available in the United States from the Fish-Schurman Corporation, 74 Portland Road, New Rochelle, N.Y., and the Swiss G.A.B. Interference Filters from the Photovolt Corporation, 95 Madison

work, the high percentage of transmission makes it possible to use them in photomicrography when necessary to record or publish results of visual work accomplished through their use. They are quite expensive as compared with wide-band filters of the Wratten or Schott Jena Glass types.

Another development of value in this connection is the High-Power Monochromator made by Leitz. Monochromators have always been the preferred method of securing monochromatic light at any given frequency, their one drawback in photomicrography being the low intensity of the light available. The Leitz Monochromator shown in Figure 149 can be employed with an arc lamp if desired, and thus a high light intensity can be secured at any desired wave length.*

FLUORESCENCE MICROSCOPY

Another type of work closely associated with ultra-violet light is fluorescence (or luminescence) microscopy. The invisible short waves of the ultra-violet region are able to set up sympathetic vibrations of longer wave lengths when they fall on certain substances. When these longer wavelengths are within the vibration range of the visible spectrum, they become evident to the eye and the substance emitting them appears luminous. This phenomenon is known as fluorescence when the visible light effect ceases quickly after the exciting rays are cut off, and as phosphorescence when the reaction persists for an appreciable time longer.

Only a limited number of substances fluoresce to a degree sufficient to make them of interest from the microscopical point of view. Minerals and chemicals furnish the most brilliant examples, but instances are not lacking in the animal and vegetable kingdoms. Fluorescence offers an additional means of identifying certain elements, tissues, etc., and also another method of differentiating various micro-structures.

Avenue, New York 16, N.Y. The Bausch & Lomb Optical Company, Rochester, N.Y. have also recently put out a set of Interference Filters. Information regarding all three of these can be obtained from the companies carrying them.

* The author has used for many years one of the original Hartnack Monochromatic Condensers manufactured by Zeiss. This mounts directly in the substage of Zeiss microscopes in place of the regular condenser. It is a fine piece of apparatus for examining objects in any color, but of course is entirely inadequate for photographic purposes. It is unfortunate that similar equipment is no longer available for visual work.

In recent years, considerable progress has been made in fluorescence microscopy through the treatment of tissues with fluorescing chemicals for which there is a selective effect in various structures. Although no change may be apparent to the eye, the effect under ultra-violet light is analogous to differential staining in ordinary microscopical preparations.

Depending upon the type of work being done, fluorescence microscopy may, or may not, require considerable special apparatus over that needed for ordinary work. In the first place, some form of lamp rich in ultra-violet rays is essential. The preferred sources are either an arc lamp (preferably with iron electrodes) or a quartz mercury vapor lamp, or both, since the effects are somewhat different with each. There are now on the market special ultra-violet bulb lamps which suffice for visual work but do not possess sufficient intensity for photomicrographic work.

Fluorescence microscopy can be divided for consideration into two groups — that with incident light, and that where transmitted light must be used. The systems of illumination do not, in theory, differ from those where visible light is employed. The ultra-violet is secured by the use of either Kodak Wratten, Jena, or Corning glass ultra-violet filters. As most optical glass transmits considerable ultra-violet, the glass lenses (condensers, etc.) can be employed with fair results.

Where maximum illumination is desired, however, replacing all the glass in the illuminating system with similar elements of quartz materially increases the brilliance of the fluorescent effect, since the absorption of ultra-violet through several thick glass lenses is considerable.

For visual work, no further change in the illuminating system is necessary. With only ultra-violet light striking an object, in both incident and transmitted setups, the field should appear dark when no fluorescing object is in place, but wherever fluorescence occurs, the effect should at once be evident. But photographs cannot be taken under these conditions, since ultra-violet light, to which the eye is insensitive, can be both transmitted and reflected by the object, and if allowed to reach the plate would register an image of its own.

It is imperative, therefore, that every bit of ultra-violet light be prevented from acting on the plate. This is accomplished by the use of a filter placed over the front of the objective or somewhere in the system, between the object and the plate. Unmounted sheet

gelatin filters are often advantageous for this purpose, as their thickness does not interfere with the performance of the lenses. The exact filter to be chosen depends somewhat on the ultra-violet filter employed. It is essential that there be no overlapping of the two. Usually the Kodak Wratten #8 (K₂) suffices. For strictly transmitted-light work, cover glasses are available which act as filters to remove the ultra-violet, but when these are used, there is no opportunity to examine the object by means of incident light projected onto the top surface.

A supersensitive panchromatic plate or film should be used for the photography of fluorescent effects so as to obtain full rendition of the light emitted, which frequently contains considerable red. Also, the total light available is very feeble and a long exposure is necessary even with fast plates. For practically all types of fluorescent work only low powers can be used, as otherwise exposure times may extend into many hours. Because of this condition, photomicrographic work in the field of fluorescence microscopy has been, up to the present, of extremely limited application.

MONOCHROMATIC ULTRA-VIOLET PHOTOMICROGRAPHY

Photomicrography with monochromatic ultra-violet light is radically different from that previously described, where a fairly wide band is employed. In monochromatic work, since a single wave length is utilized, special optics can be designed to give ideal correction at the specific wave length, without regard to meeting conditions throughout a wide spectral region. The primary purpose in working with monochromatic ultra-violet light is to secure greater resolution, for, as pointed out in Chapter 1, the shorter the wave length of the light employed, the larger the number of lines per inch which can be resolved.

Three practical methods have been developed to utilize a monochromatic ultra-violet light source. The simplest, and at the same time least expensive, is that of Bausch & Lomb which utilizes the 3650-3655 a.u. lines in the mercury vapor arc. The manufacture of this outfit has been discontinued in favor of their new, improved (and much more expensive) equipment to be described later. However, there are many of these older outfits in use and giving good service; hence outlining this method appears justified.

The special objectives, instead of being corrected over a wide band,

are designed to be chromatically and spherically corrected at two wave lengths only — the 3650 a.u. ultra-violet line and the 5460 a.u. green line of the mercury vapor lamp. To meet such correction is relatively easy, whereas, if a lens were required to function equally well at all points between these limits, compromises in the computation of the lens would have to be made which would lower its performance at the desired ultra-violet wave length. The greatest single obstacle to overcome in the technique of photography outside

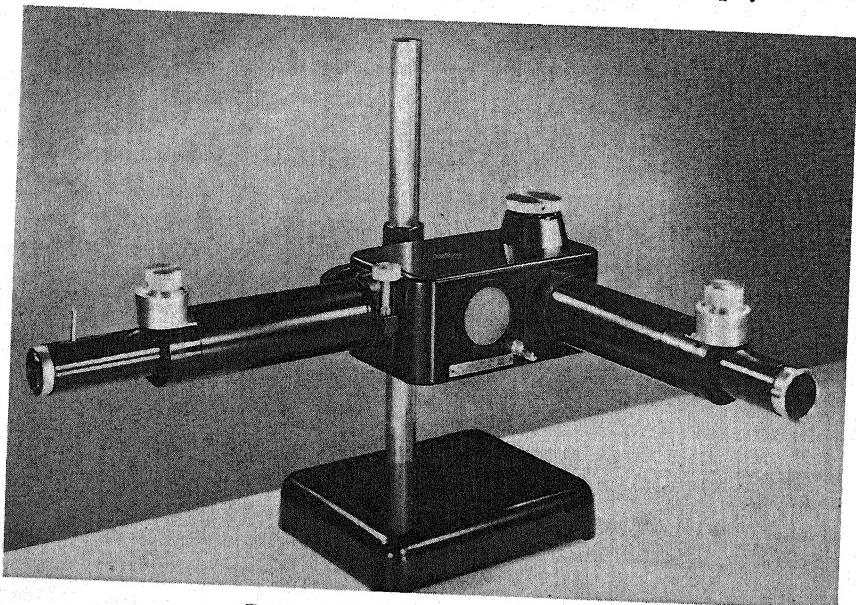


FIG. 149. The Leitz Monochromator

the visual range is the difficulty of focussing the object on the plane of the sensitized plate.

With the Bausch & Lomb special lenses, the focussing is accomplished by the aid of the green (5460) line and a filter which passes only this line. When the green image is in focus, the ultra-violet image formed by the ultra-violet (3650) line is likewise in focus, so that changing filters is the only additional operation necessary in order to make an exposure with ultra-violet. The green and ultra-violet filters are furnished as part of the complete Bausch & Lomb ultra-violet photomicrographic outfit, which is illustrated in Figure 150.

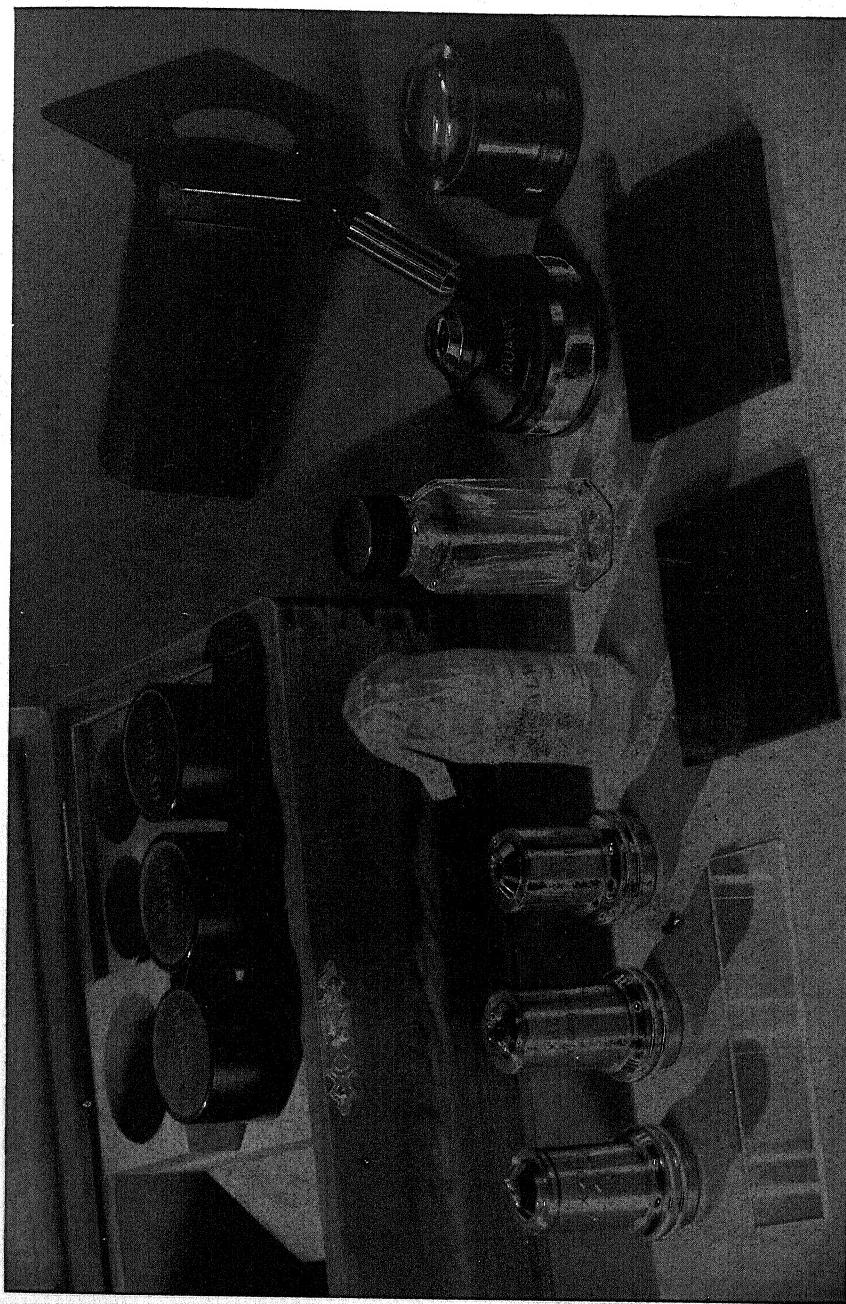


Fig. 150. The old Bausch & Lomb Outfit for Ultra-Violet Photomicrography with the Mercury Arc

The immersion fluid employed with the high-power oil-immersion objective is not cedar oil (as standard for visual work) but sandalwood oil.

When the highest possible performance with the oil-immersion lens is desired, it is a good idea to make a check on the exact focal point of the ultra-violet as compared with the green. This check is made by the method described for ascertaining the divergence of the ultra-violet focus when working with ordinary objectives, outside of the visible range. The divergence, if any, will be found to be slight, and a factor only when photographing such objects as the markings on *amphipleura pellucida*, or a ruled grating, for maximum resolution. Because of the likely small divergence, the steps in the test exposures should be of the order of $\frac{1}{4}$ to $\frac{1}{2}$ micron, and it is not necessary to extend the test beyond two microns on each side of the green focus.

Comparative tests on a given specimen (e.g., of *Amphipleura pellucida*) reveal at once the great increase in resolution obtainable with the short wave length.*

The transmission of very thin films of the majority of mountants for the 3650 a.u. line is such that most objects can be photographed, regardless of the nature of the mounting medium. But there are some limitations. For instance, realgar, the best medium for finely marked diatoms from the standpoint of refractive index differentiation, possesses such strong absorption in the ultra-violet region that it is ruled out for all ultra-violet work. The nearest high index mountant which can be used is Hyrax. This absorbs considerable ultra-violet, but can be used if a sufficiently long exposure is given.

The alternate method of photographing with a single line in the ultra-violet is one developed many years ago by the Zeiss Company, although without its ever experiencing an extended demand. The reasons for this are several. In the first place, it is primarily a research tool, intended largely for one specific purpose — acquiring further knowledge of microstructures through increased resolution. It does give remarkable improvement in resolution under the limited conditions where it can be used, but there is no record of a single new

* It is not feasible to make direct comparisons, even of the same species of diatom (*amphipleura*) when photomicrographs have been taken from different slides, because of the wide divergence among the individual diatoms. This is especially true when they are not from the same locality. Even in a single slide of specimens from the same locality, there will be found some difference in the degree of sharpness of the markings between various individuals when oriented in the same position. Ideal specimens showing the dot structure sharply defined are rare.

fact brought to light through the superior resolution it provides. The equipment is expensive; its cost can therefore be justified only in occasional instances, since its use is very limited. Further, the technique of its operation calls for knowledge and experience far beyond that possessed by the rank and file of microscopical workers. Regardless of all this, it is still entitled to rank as one of the highest of microscopical developments, and other uses for it have materialized, which entirely eclipse the original idea of increased resolving power, *per se*.

There are but few of these outfits in use in the United States, and in view of Bausch & Lomb's improved monochromatic outfit it is doubtful if there will be any further demand for them. World War II stopped their production.

In theory the apparatus is quite simple. By the use of a high-voltage electrical discharge across electrodes made from a metal possessing widely separated but strongly spectral lines, in the ultra-violet, a suitable source of light is obtained. The metal chosen for the purpose is cadmium, which has several strong lines, the most suitable being that at 2749 a.u. This is far below the transmission limit of ordinary and optical glass, such as that employed for microscopical purposes, and hence is useless with regular microscopical lenses. Moreover, it cannot be passed through an ultra-violet filter of the Kodak Wratten or Jena types, such as those used with the Bausch & Lomb outfit. Recourse must be had to crystal quartz for every portion of the optical train, including the collimating condenser, microscope substage condenser, all objectives and eyepieces, and a quartz prism substituted for the mirror. In addition, the isolation of the 275 m μ line (as the 2749 a.u. line is usually called) must be accomplished by means of quartz prisms which separate the lines sufficiently far that only the 275 line can enter the quartz substage condenser of the microscope.

The microscope objectives are highly corrected for the 275 line, but as long as the light is monochromatic, other lines can also be used. The most common substitute source is a group of four lines at 2791 — 2796 — 2798 — 2803 a.u., in the magnesium spectrum. Since there are so many of these powerful lines together, the illumination is much more intense, although theoretically the performance of the objectives is not of so high an order, the light not being strictly monochromatic. The relation of the cadmium and magnesium lines is shown in the spectrogram of these metals (Figure 151).

As no visible light is present, preliminary location of the desired field is secured by the use of an auxiliary light reflected into the substage from the face of one of the monochromating prisms. For rough focussing with the ultra-violet light a special viewing device termed a finder is placed over the eyepiece. This contains a fluorescent screen and a magnifier for viewing the image formed on the screen. When suitably adjusted the image is in focus at a fixed projection distance when the fluorescent image is sharply in focus in the finder.

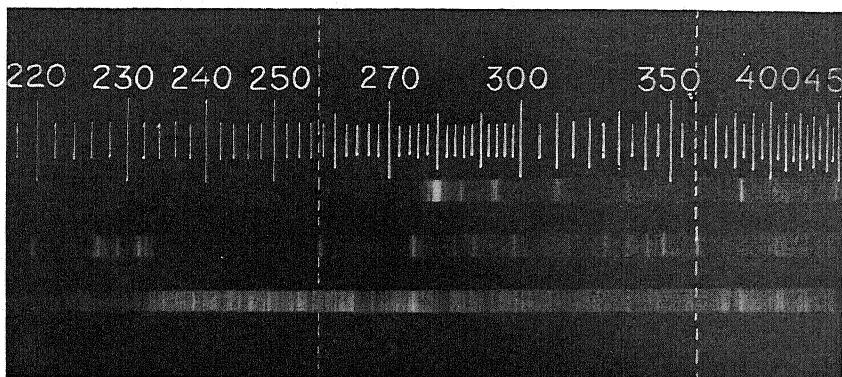


FIG. 151. SPECTROGRAM OF CADMIUM AND MAGNESIUM

Showing the spectral lines of magnesium (top), cadmium (middle), and iron (bottom) in the region between 2200 and 4500 a.u. The single cadmium line at 2750 and the several magnesium lines at 2800 are those chosen for photographing in the ultra-violet with the Zeiss outfit.

Best results are obtained when the power source is alternating current, using a stepup transformer ($\frac{1}{2}$ K.V.A.) operating at 10,000 to 15,000 volts, in combination with a tuned circuit. Where only direct current is available an electrolytic or mercury interrupter must be provided, in combination with a high potential spark coil. The latest form of the outfit, with fixed electrodes, is shown in Figure 152. Since its introduction the apparatus has undergone continued change of mechanical design, although the optics remain substantially unchanged.

Many of the limiting conditions which circumscribe the use of this apparatus are associated with the poor transmission of most substances for a wave length of $.275 \mu$. Glass, all common mounting media, the majority of tissues, all stains, most inorganic chemical compounds, and

other substances in which the microscopist is interested, appear opaque in this ultra-violet region. Hence no ordinary microscopical preparation can be studied by this apparatus.

Objects must be mounted on quartz slides, in a medium such as colorless petroleum oil (Nujol) or glycerin, and covered with quartz cover glasses. The small quartz slides, one of which is shown on its metal carrier, in Figure 153, cost about \$2.50 each and the $\frac{1}{2}''$ circular

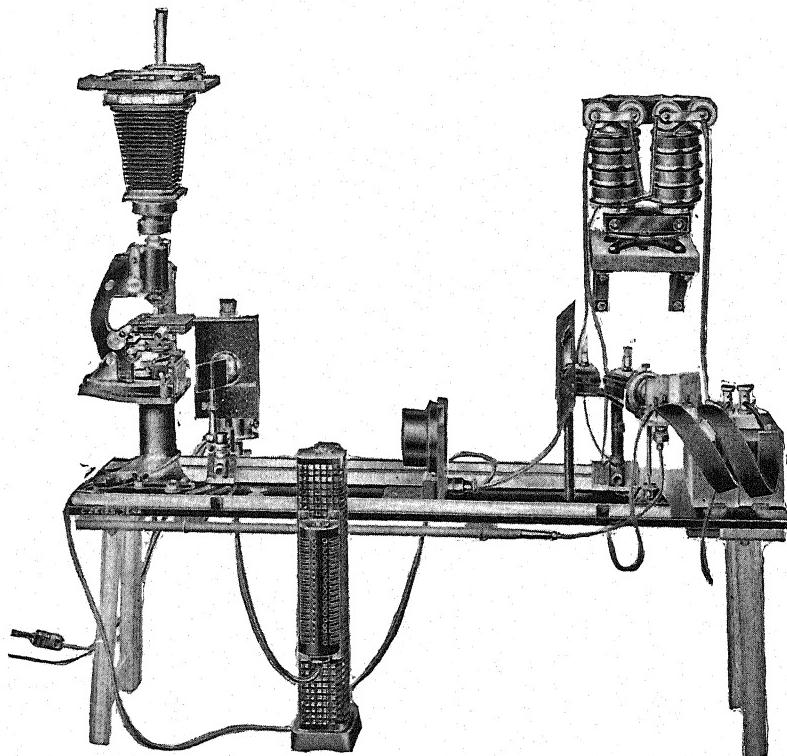


FIG. 152. Zeiss Ultra-Violet Equipment with Fixed Electrodes

covers are \$3.00 each. It is not surprising, therefore, that substitutes, in high-silica glass, have recently been developed. These are far cheaper and satisfactory for many types of work. Their transmission is around 75 per cent of quartz, for the 275 $\mu\mu$ line, but falls off rapidly below this.

From a practical standpoint, the greatest value of this ultra-violet equipment is in the differentiation of various microconstituents

through the degree of absorption present. For instance, if one had a mixture of powdered glass and amorphous (fused) silica, the proportion of each could be quickly determined: the glass would photograph black, while the fused quartz particles would be transparent.

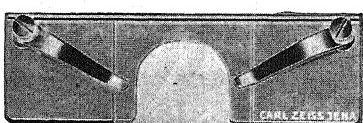


FIG. 153. Rock Crystal Object Slide,
with Metal Carrier

It is possible by the use of this apparatus to differentiate between the cytoplasm and nucleus of unstained, living cells, since the nucleus strongly absorbs ultra-violet. This opens up a means of studying morphological changes taking place in the living cell, and the transitional

steps between living and dead protoplasm.

Although the entire operating technique of this apparatus calls for the consideration of many details which cannot be discussed in our limited space, most of the general processes are similar to those already considered. As with other cases where the image cannot be directly observed on the ground glass, tests for critical focus have to be made. Tests to determine the correct exposure obviously must be made at the beginning of the work, after which computed exposures can be relied upon for variations in magnification. Computed exposures, however, are of no value when one must differentiate between two constituents through their absorption characteristics, if the percentage of transmission in both is of a low order. Plate XLVI in Chapter 10 is an example of this sort. Very slow, fine grain, non-color-corrected plates, or films such as Process types are best suited for work with ultra-violet light.

The third type of ultra-violet equipment is Bausch & Lomb's newest and most versatile outfit. The basis of it is their improved grating Monochromator, which, in combination with quartz optics, provides for the utilization of any desired wave length in both visible and ultra-violet regions (Figure 154). The principle on which the monochromator operates is shown diagrammatically in Figure 155. Light is projected through a slit to a plane 45° mirror, then against a concave collimating mirror which in turn projects it to the grating. From here the dispersed light is returned to the collimating mirror at an angle which causes it to be reflected to the exit slit and into the microscope. The desired wave length is secured by rotation of a drum controlling the positioning of the grating. The drum is calibrated in angstrom units for selecting the wave length wanted.

Three types of lighting equipment are available, a High-Intensity Mercury Arc, a Ribbon-Filament Lamp for work in the visible spectrum, and a High-Pressure Hydrogen Arc Illuminator giving a continuous spectrum in the ultra-violet region. (While it is not suggested or furnished, it appears that an iron arc lamp with special alloy iron electrodes would also be a valuable adjunct for high-intensity lines in the ultra-violet, and also a cadmium or magnesium

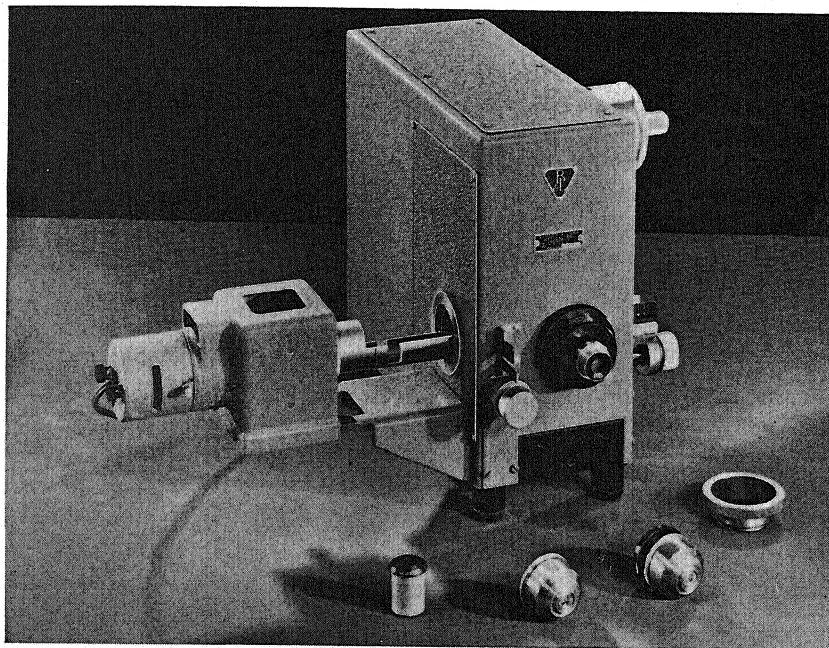


FIG. 154. Bausch & Lomb Grating Monochromator and Ultra-Violet Optics

spark outfit, operating on about 15,000 volts. With such apparatus, work could be done in the 2750-a.u. region.)

For ultra-violet work an achromatic quartz condenser is provided for the Monochromator for projection into the optical system of the microscope. In the microscope the substage condenser and objective must not only be capable of transmitting ultra-violet but must be achromatic for all wave lengths. To accomplish this the reflecting objective principle proposed in the early days of microscopy by Sir Isaac Newton has been utilized in the design of both condenser and objective (which are identical, merely working in opposite directions).

The eyepiece must have quartz optics, and the image must be picked up on a fluorescent screen for visual observation. Photography can be done with any type of microscope stand. The Monochromator and complete setup for ultra-violet work are shown in Figure 156.

The Bausch & Lomb Monochromator, while designed especially

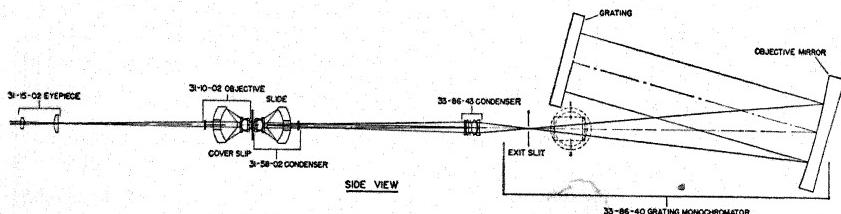
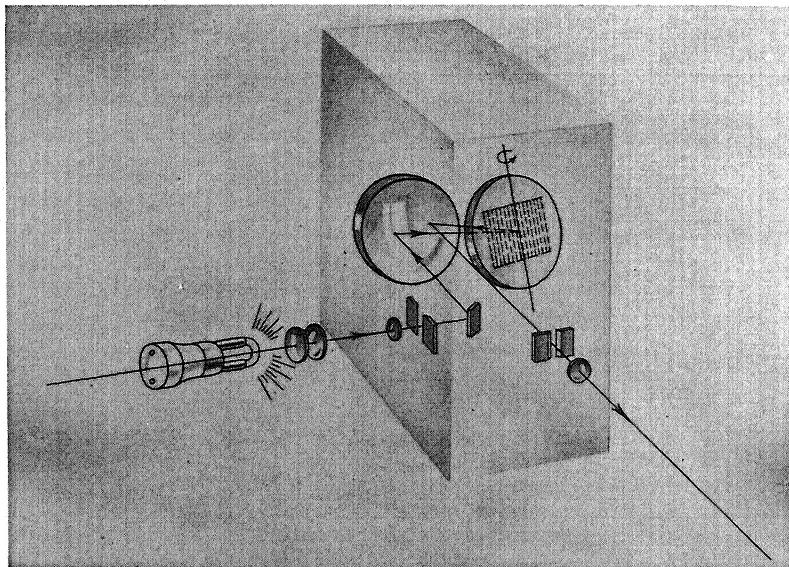


FIG. 155. (Top) A Schematic Diagram of the Bausch & Lomb Grating Monochromator. (Bottom) The Path of Light Rays in the Bausch & Lomb Monochromator

for ultra-violet work, is also adapted for use in the visible spectrum when employed with a ribbon-filament lamp or other high intensity source. However, if work only in the visible range is to be done, one should balance the initial equipment costs of this outfit against a monochromator of the Leitz type and narrow-band filters of the

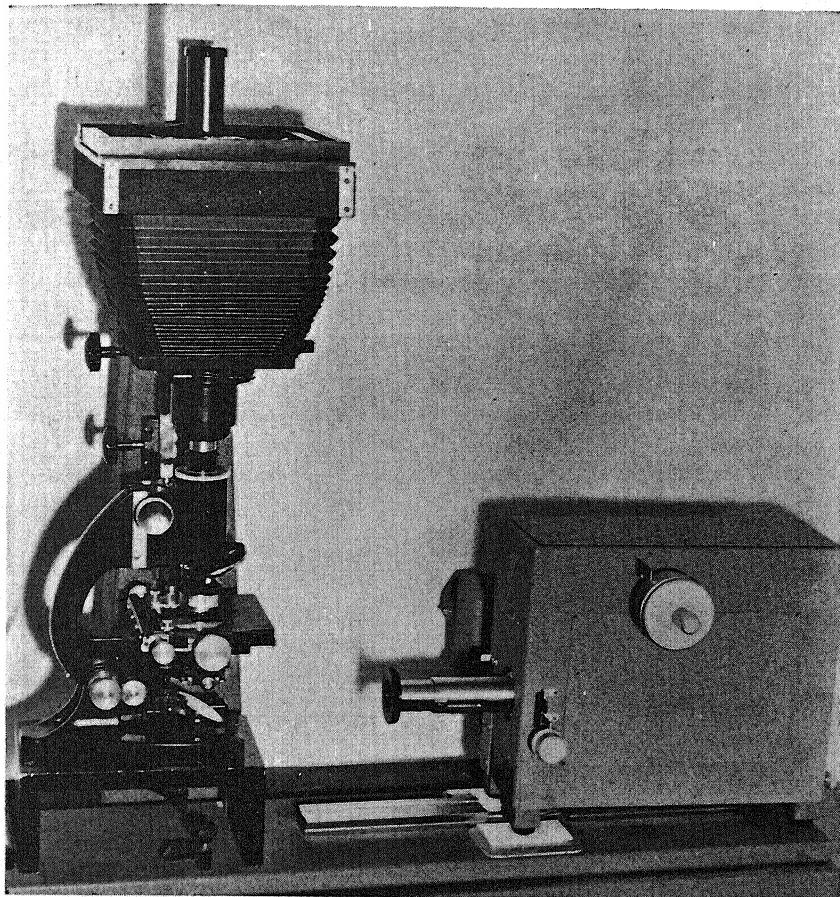


FIG. 156. The Bausch & Lomb 250-mm. Grating Monochromator Research Microscope with Ultra-Violet Optics

required regions before deciding which is the more suitable and economical. There is, of course, no question involved when ultra-violet work is contemplated.

MOTION-PICTURE PHOTOMICROGRAPHY

Though the field of motion-picture photomicrography is considerably restricted, it is of extreme importance for revealing what takes place under certain conditions. Original applications of motion pic-

tures through the microscope were confined largely to displaying living organisms — protozoa, etc. — in water. Such pictures were welcome additions to various educational films, in the standard 35 mm. class.

Recent motion-picture work has been largely confined to the 16-mm. class and microscope manufacturers have developed apparatus and accessories for it. The smaller film possesses several advantages over the standard size, for photomicrographic work. Chief of these is the possibility of using a less intense light for the same field area

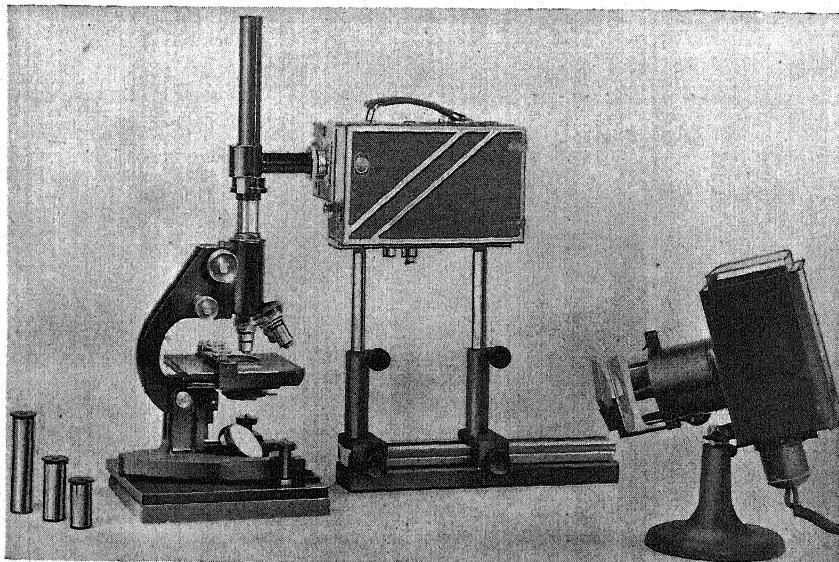


FIG. 157. Bausch and Lomb Outfit for Motion Picture Photomicrography

covered, since the light on the film is inversely proportional to the area of the two sizes, i.e., about five times as great on the 16-mm. film. Thus for equivalent exposure a light of one-fifth the intensity will suffice. Excessive concentration of light, with its attendant heat, is objectionable in the case of many minute organisms and delicate living tissues. Death frequently ensues very quickly under intense light and heat and the reactions which may have been photographed under this condition cannot be considered typical.

Among the specific classes of work especially adapted to motion-picture photomicrography can be mentioned (in addition to the photo-

graphing of living micro-organisms): studies of blood circulation and the function of the various blood cells in rebuilding tissue, cell proliferation (mitotic processes, etc.), crystal growth, chemical reactions, Brownian movement — in fact, anything where changes in structure or relationships can be shown to occur over a period of time.

The camera required for motion-picture photomicrography must be a standard commercial model from which the lens can be removed. It is not possible, as in other types of photomicrographic work, to connect the microscope directly to the camera, since it is necessary to have the field being photographed under observation at all times. The intermediate fitting, known as an observation eyepiece, is therefore an important and essential portion of the equipment. For this reason, it is a better policy to purchase the outfit as supplied by the various manufacturers than to attempt to construct it of nondescript parts. The Bausch & Lomb outfit is illustrated in Figure 157. It is designed to operate with various models of Ciné Kodak cameras. Any standard microscope can be used, but special eyepieces are required. When a rapidly moving object that is apt to leave the field of vision is being photographed, it is essential that the microscope be equipped with a mechanical stage. It is frequently necessary to be constantly manipulating both the fine-focus adjustment and the mechanical stage while such objects are being taken.

Some of the rapidly moving organisms may be photographed with "slow motion" (i.e., 64 frames per second) with advantage, but the light intensity should be increased accordingly. It is possible to slow down the motion of organisms considerably by adding to the water a viscous medium, such as gelatin, egg albumen, solution of slippery elm bark, gum arabic, etc.

When the problem concerns the photographing of changes taking place so slowly that no movement can be observed over a considerable period of time, and a period of many hours, or even days, may be involved in the total cycle to be depicted, it becomes necessary to devise special apparatus for the purpose, or secure one of the outfits which have been made available by some manufacturers. A very practical yet simple outfit is manufactured by Silge & Kuhne. It is illustrated in Figure 158. It can be used with any type of camera. What is required is a clock-driven timing device which will turn on the light and make a single-frame exposure at predetermined periods. In other words, instead of making exposures at the rate of 16 per

second, they may be one per second, one per minute, or even one per hour in extreme cases. It is the opposite of slow motion, since when the frames are shown at the normal rate, what occurred in many

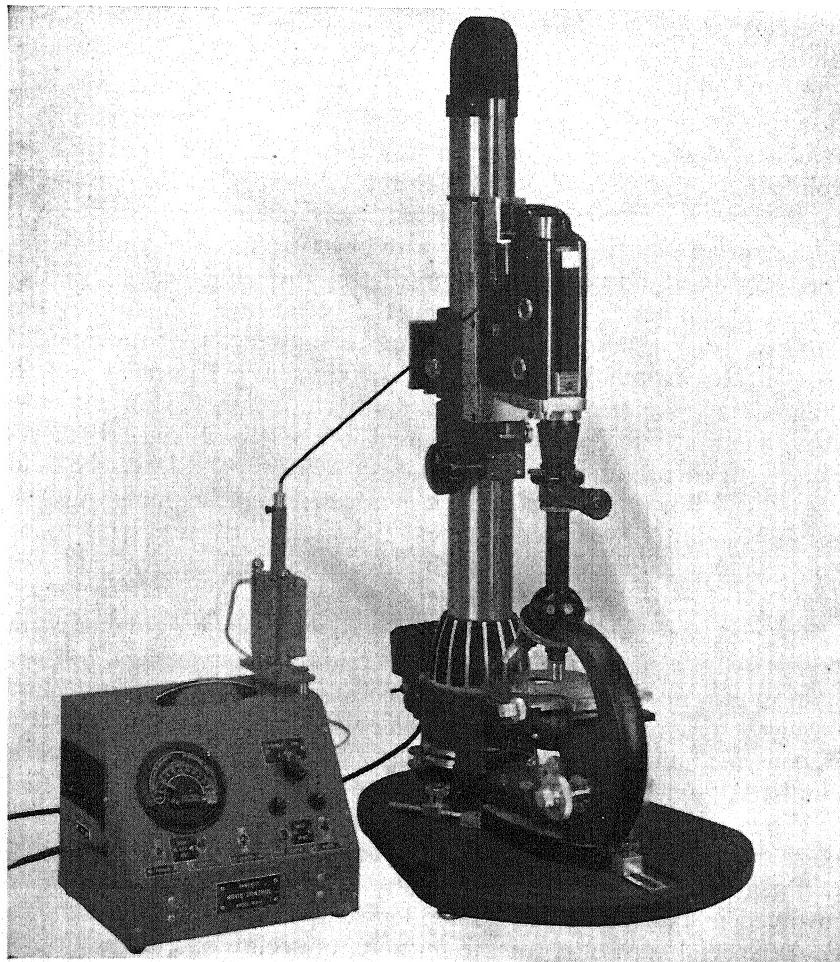


FIG. 158. Silge & Kuhne Outfit for Time-Lapse Photomicrography

hours may be shown in a minute, or less. Knowing the time interval between exposures, one can compute the elapsed time in any particular cycle, by counting the number of frames from start to finish.

Details of setting up and operating may vary considerably, depend-

ing upon the make of equipment secured, and hence recourse must be had to the instruction booklet issued by the firm manufacturing the outfit. The fundamentals of general photomicrography as to securing critical illumination, use of filters, determining exposure times, etc., apply equally well to motion-picture work.

PHOTOMICROGRAPHY IN COLOR

Any color process used in ordinary photography is equally adaptable to photomicrography. Some, however, are subject to limitations which make it more difficult to secure correct color rendition, though this may not be so serious a condition as when portraying natural colors, since the primary purpose of color in microscopic work is differentiation of structure.

Modern advances in color films have made many of the earlier processes obsolete, even though some of them offered advantages in simplicity of operation for the occasional worker over those now in use. From a historical standpoint earlier processes for making color plates deserve brief mention, especially as they serve to illustrate the radical improvements which have been accomplished and the line of development through which these have come.

The basis of the single-process type of color plates lay in the use of an underlayer of color applied to the glass, over which an ordinary panchromatic emulsion was placed. In the Lumière Auto-chrome this color layer was composed of cornstarch grains dyed in the three primary pigments, blue, green, and red, in the proper proportions to yield an approximation of white light under a reversal process. These were attached to the glass by means of a varnish layer. The minute size of the grains resulted in a total of around 3,000,000 colored areas in a single square inch, the spaces between them being filled with carbon black. To use these plates it was only necessary to expose them as though they were black and white, except through the back of the plate so that the light must pass through the color screen to reach the emulsion. They were then developed fully, the exposed and blackened silver was dissolved out, and the un-exposed silver remaining in the emulsion was exposed to a bright light. After this exposure the plate was redeveloped. The result is a positive image, viewed as a transparency.

The Agfa color process was similar, the essential difference being that the color screen was composed of dyed particles of gum arabic

instead of starch grains, warmed until melted, on the plate. In transparency, the Agfa plates were a considerable improvement over the Lumière plates. The appearance under the microscope of the color screens of these two processes is shown in Figure 159.

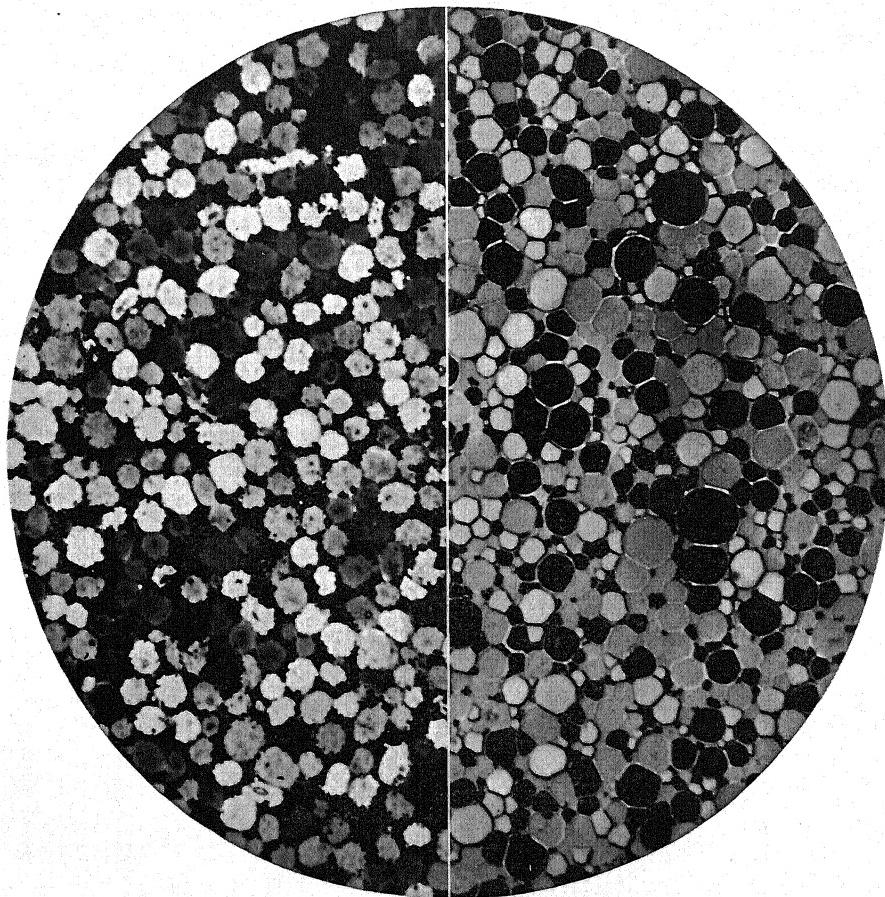


FIG. 159. PHOTOMICROGRAPHS OF LUMIÈRE AND AGFA COLOR SCREENS

The color screen of dyed starch grains of the Lumière Autochrome plate is on the left; the screen of the Agfa plate is on the right. Both are magnified 300 \times

The Dufay process differed from the Lumière and Agfa processes in that the color screen was mechanically printed in minute squares running about 1000 to the inch (1,000,000 to the square inch). The

technique of processing was similar to that for Lumière and Agfa plates.

The Paget color process and its later revamped version, the Finlay

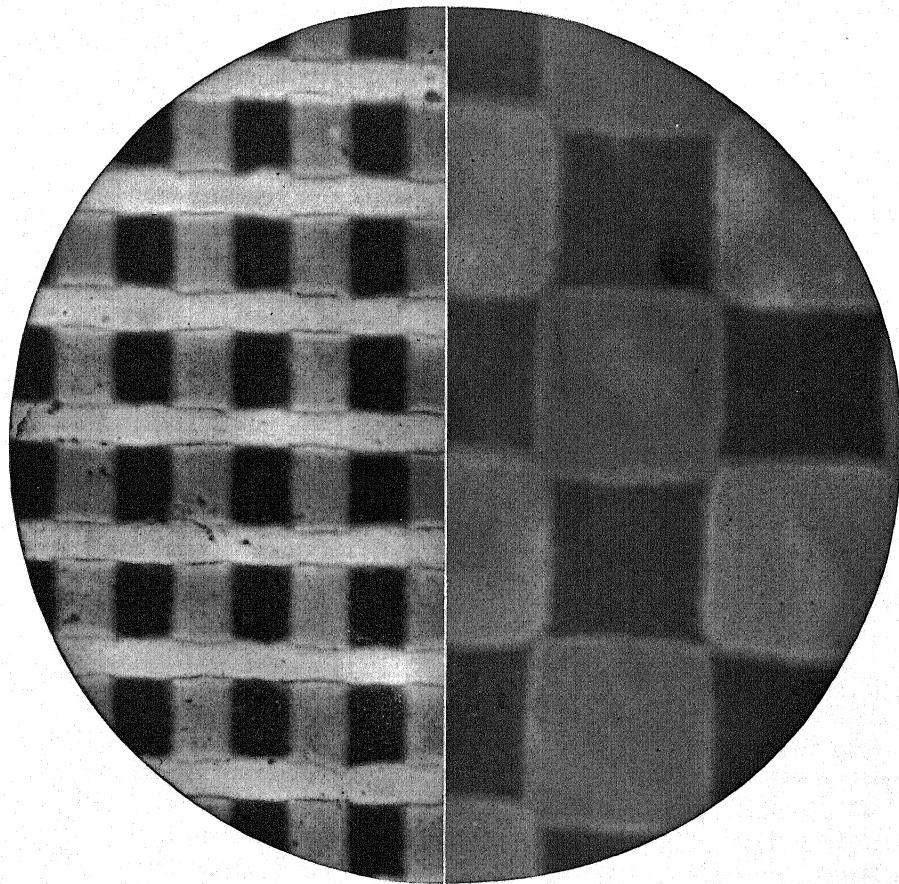


FIG. 160. COLOR SCREENS OF THE DUFAY FILM AND THE FINLAY PLATE

Left, Dufay film. *Right*, the Finlay (old Paget) plate. Since both are at a magnification of 300 \times , they are directly comparable with the Lumière and Agfa screens in Figure 159

color process, differed from the Dufay in that the printed color screen was on a separate film which was placed next to the sensitized film (of any panchromatic plate) in the plateholder and the picture was

exposed through it. Thus the developed negative consisted of a réseau of fine squares in varying degrees of black and white. Positives (as many as desired) could be made from the single negative and bound up as transparencies with color screens identical with the taking screen, after careful superposition of the proper squares with their corresponding colors. Figure 160 shows comparative views of the Dufay and Finlay screens at the same magnification as the Lumière and Agfa screens.

Separation-Negative Processes

For many years the separation-negative type of color pictures was popular for ordinary photography and would yet be in vogue had not color processes of the Kodak Kodachrome type replaced it. The basic principle involved is the taking of three negatives (on panchromatic film) of the same subject, through blue, green, and red filters. From these, prints are made on transparent gelatin film, which is then toned in the complimentary colors. Those three-toned films are then superimposed on a white paper support, the result being a reproduction of the object in approximately natural colors.

The time and trouble involved in this type of color pictures mitigated against its general acceptance for photomicrographic work (since three separate exposures must be taken for each picture), although the Defender Chromotone process was ideal when paper prints were desired for reproduction purposes. The problem of three exposures in sequence did not arise in ordinary photography, since cameras were designed that could take all three exposures simultaneously, an important requirement when movement of the object was involved.

This three- or four-negative process must still be employed in the reproduction of color pictures for magazines, books, and similar work. (The finest printing processes in reproduction of color pictures involve the addition of a fourth printing in black, which is not required in original color photography.)

It was inevitable that sooner or later means would be found to take all three colors on a single film at a single exposure. This was brought about by the Eastman Kodak Company through the introduction of Kodak Kodachrome.

Kodak Kodachrome

The general photographic public was first made aware of the advantage of Kodachrome through the use of 8-, 16-, and 35-mm. roll film. This was followed by the introduction of professional sheet film in various commercial sizes. This latter soon offered the finest expression of color rendering available to the photomicrographer. Through the adaptation of 35-mm. minicams to the microscope, 35-mm. Kodak Kodachrome soon became popular with amateur microscopists. The most serious disadvantage of this film for microscopical work lay in the necessity of sending the exposed film to the Eastman Kodak Company for processing. This involved not only a considerable delay but a degree of photomicrographic knowledge adequate to insure freedom from failures in exposure. On the other hand

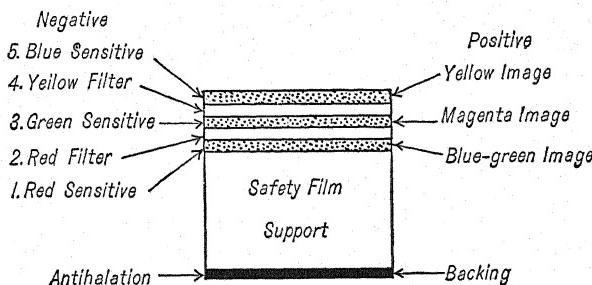


FIG. 161. A Cross Section of Kodachrome film

the actual work required was less than with any other color process.

Figure 161 shows diagrammatically the principle upon which the three-color separation depends. Since the three images are produced in the processing through dye coupling, no mosaic patterns are present and the only graininess evident is that of the silver emulsion, which has been reduced to almost zero. Thus the enlargement possible in viewing or projecting is enormous. The development processes involved in the production of the final transparency require numerous steps of multiple dyeings, washings, etc. — all of which must be carried out at controlled temperatures, definite pH control, and accurate time cycles. Thus factory processing was imperative in the early stages of the introduction of Kodachrome color pictures.

A simplification of the Kodak Kodachrome film resulted in the

introduction of Kodak Ektachrome film in sheet film sizes. This film could be processed by laboratories outside the Eastman laboratories. Once this film was standardized for commercial processing, the manufacture of Professional Kodak Kodachrome was discontinued and Kodachrome film was supplied in roll-film only. For a while the processing of this roll film was continued at Eastman laboratories, the cost of the processing being included in the price of the film. Even this practice has since been discontinued and the price of the film reduced; thus unless one desires to undertake the involved development processing himself, this work must now be turned over to commercial processing companies.

In addition to Kodak Kodachrome and Kodak Ektachrome film, which are of the reversal type and finished as transparencies, the Eastman Kodak Company also make Kodak Kodacolor and Kodak Ektacolor films. These differ in that they are negative films, processed in supplemental colors from which positive prints can be made, either as transparencies or paper prints. Conforming to the practice established for Kodachrome and Ektachrome, Kodak Kodacolor is supplied in standard roll-film sizes while Kodak Ektacolor comes in sheet film of commercial sizes up to 8" x 10". When it is known that a considerable number of copies of a single picture are desired, Ektacolor offers the best solution for color work, although to date very little of either Kodacolor (in the 35-mm. size) or Ektacolor has been adopted for general photomicrographic use.

All films are furnished in two types, one for daylight and one for artificial light. For photomicrographic use the latter is preferred; otherwise compensating filters are required. Ordinarily filters are not required for artificial (tungsten) light, provided the latter has a color temperature of 3200°K. When artificial light of other types or temperatures is employed, compensating filters are required. The accompanying table of color temperatures for various light sources shows the Kodak Wratten filters recommended for compensation to yield correct color values in the pictures.

Minor variations in every step in the manufacture and processing of color film, in spite of rigid controls, necessitate factory tests on each emulsion batch produced. Accordingly, data regarding variations in speed and possible color compensation by means of special balancing filters are enclosed in each package of film.

Other companies, like Ansco, have put similar color film on the market; hence one is not limited in his choice of film. There is a

COLOR TEMPERATURE (AT LAMP) OF VARIOUS LIGHT SOURCES^a

Lamp	Voltage	Amps.	Color Temp., °K			Filter Recomm.
			Av.	Min.	Max.	
Ribbon filament	5.5 ^b	17.7(ac)	2848 ± 54	2769	2962	
Ribbon filament	6.8	—	3000	2911 ± 27	—	
Leitz Monla	6. ^c	16.5	3000	3012	82 + 82A	Two 82B 82C
Coil filament	5.2	—	3050	—	82A	82B + 82C
Coil filament	5.5	—	3200	—	—	Two 82A 82B
Coil filament	6.1	18.0	3200	—	—	Two 82A 82B
Zirconium arc (100-watt)	118	1.8	3230 ^d	—	—	Two 82A + 82B 2B + 82B
Carbon arc (Line)	4.5	—	3645 ^e	—	—	2B + 82A + 82B
Carbon arc	10.	—	3820 ^e	—	—	

^a From a table furnished by Mr. R. P. Loveland of the Eastman Kodak Company.^b Voltage at bulb. Chosen as representing an average frequently encountered when no ammeter or voltmeter is used.^c Needle at red line on meter supplied with lamp.^d The standard power supply made by the George W. Gates Co. was used.^e Excessive ultraviolet (near).

slight difference in color rendering in the various products available.

As a rule, photomicrography in color is not so exacting as in ordinary photography as long as there is sufficient differentiation in various structures represented. However, there are occasions when it is important to portray certain stains in their true color. This often imposes a problem, since the dyes used in the film cannot express the correct color of the stains. To remedy this condition for certain important stains, compensation can be effected by special filter means.

The value of color photomicrography lies in its application to three general types of objects — those possessing inherent natural colors, those artificially colored (histological and pathological sections, fibers, etc.), and those where polarized light or fluorescence are employed to different structures. One of the more recent applications of polarized light is in the study of metals by means of reflected polarized light.

For those wishing to excel in color photography and photomicrography it is advantageous to study publications which have been prepared to cover the fine points involved in the correct rendition of color and the means of overcoming limitations in the color films, the exposure times, and the processing. Also available are instructions for processing, should one wish to do this work oneself. The following publications of the Eastman Kodak Company are of value and procurable from camera stores.

Photography Through the Microscope, 2d edition. Price 75¢.

Kodak Color Films. Price 75¢.

Kodachrome and Ektachrome Exposure in Photomicrography.

Photomicrography of Stained Slides with Kodachrome and Ektachrome Films.

Kodak Wratten Filters for Scientific and Technical Use. Price 75¢.

Kodak Films. For black and white, but much data on exposure curves; price 50¢.

Metallography in Color, by R. P. Loveland.

Quality and Quantity of Illumination in Color Metallography, by R. P. Loveland.

These two books are published by the American Society for Testing Materials.

Color Photomicrography in the Laboratory, by R. P. Loveland, in *Analytical Chemistry*, Vol. 21, page 467, April 1949.

STEREOSCOPIC PHOTOMICROGRAPHY

Instances constantly occur in microscopy where it is a difficult and at times well-nigh impossible task to interpret properly a structure depicted in a single photograph. The value of stereoscopic pictures which can be examined in an ordinary stereoscope, in such cases, cannot be overstated.

Several devices are on the market for taking stereoscopic photomicrographs, but they are largely designed for low-power work. Camera attachments are available for mounting on Greenough binocular microscopes, or carrying the paired Greenough objectives. The Bausch & Lomb Co. have developed the Ortho-Stereo camera shown in Figure 162, which, with a complete set of lenses, takes pictures from full size up to 24 diameters. This apparatus, in combination with the special stereoscope designed for use with it, gives a true three-dimensional picture — that is, the object appears to the eye just as it would if uniformly increased in size, the same amount as the magnification.

Unless attention is paid to this particular feature, the magnification of the third dimension — i.e., the depth — may not actually coincide with the lateral enlargement. If the vertical dimension enlargement is less than the lateral, the height of the object will appear less, in proportion, while if the vertical enlargement is greater than it should be, the object will appear higher in proportion to its size. Since in both these conditions, however, a three-dimensional, plastic image is produced which gives a proper conception of an object and the relation of various planes in it, accurate in all things except the exact relation of height to breadth, a lack of orthostereoscopic reproduction is often not a handicap.

This is fortunate, since in some kinds of work a magnification of many hundred diameters is required, yet a stereoscopic image of some sort is essential, even if it be not possible to produce a truly orthostereoscopic condition.

As a rule the two pictures constituting a photomicrostereograph cannot be taken simultaneously, although, if some specific problem warranted the expense involved in constructing special apparatus, it could be accomplished by the utilization of one of the methods by which stereoscopic pictures can be produced. The only apparent need for such a device would be the photographing of living or moving matter. Very low-power stereos can, of course, be taken simul-

taneously with the Greenough binocular, but where the objectives alone are employed, and no eyepieces are present, the results are not very satisfactory. On the other hand, since there is little need for pictures of living organisms, and since non-motile subjects can be photographed in two separate exposures, this latter procedure is entirely satisfactory.

Stereoscopic or binocular vision, possessed by all individuals with normal eyesight, results from the superimposing of two different views of an object, into one composite picture, by the co-ordinating power of the brain. When an object is located at the distance of best vision

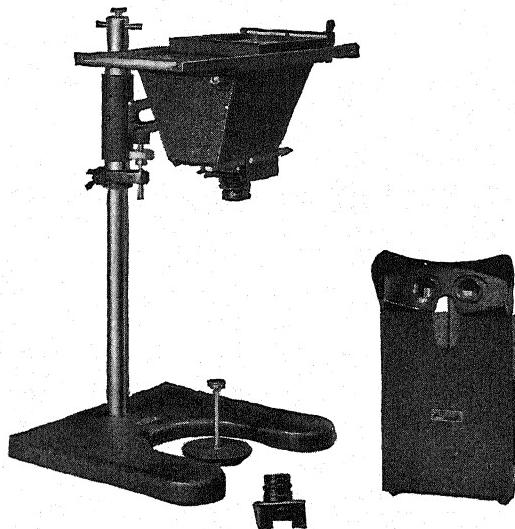


FIG. 162. Bausch & Lomb Ortho-Stereo Camera

from the eye (i.e., 10''), the parallax angle between the images is about 15° , the exact angle depending upon the interpupillary distance of each individual.

The requirement for a stereoscopic picture is that two views be taken from points separated approximately the distance between the eyes with the object seemingly located ten inches away. For an object at full size this means that the angle between the two views is around 15° . If the angle be materially less than this, the apparent height of the object is lowered although a three-dimensional effect is still obtained. Conversely, a greater angle accentuates the height.

Since, in general, it is not essential, especially for higher-power work with single objectives, that true orthostereoscopic effects be obtained, any one of several different methods can be employed with an ordinary photomicrographic outfit, to secure satisfactory results.

There is considerable separation in the angular view of an object picked up by the semicircular areas of an objective on opposite sides of the median line, so that by the simple expedient of covering one-half of the back lens of the objective to take one view, and the other half to take the second picture fairly good stereographs can be produced. A similar result is obtained by covering one-half the image at the Ramsden circle. This is the method utilized in the Abbe stereoscopic binocular eyepieces, as made by Zeiss.

Better results are obtained by a displacement of the image on the ground glass an amount equal to the interpupillary distance (i.e., about $2\frac{1}{2}''$). This method requires a camera large enough to take the two views on one plate. It also requires the use of a mask which will

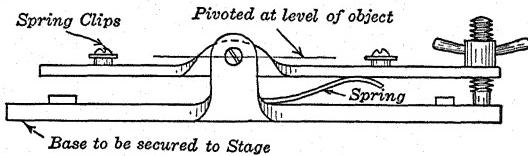


FIG. 163. Tilting Stage for Stereo-Micrographs

cover one-half the plate while the corresponding half is being exposed. The other picture is then taken by reversing the mask, making sure, of course, that the change in the position of the object has also been made.

The shifting of the image can be accomplished either by means of a shift of the object with the mechanical stage, or if a centering objective holder is available, the objective can be displaced laterally, each side of the center, the proper amount. It is preferable to locate the center of the desired area in the center of the ground glass, then shift it to the right (or left) an amount equal to one-half the desired displacement for the first view, and in the opposite direction an equivalent amount beyond the center, for the second picture.

By far the best results are obtained when a tilting stage can be used. Such an item is not available as standard equipment at the present time, but possibly will be in the near future. It can be made by anyone handy in the working of metals. A sketch of the one

designed and used by the author is shown in Figure 163. The secret of a successful design is to have the line of pivoting cross the optic axis of the microscope and at the same time lie exactly on the plane of the object. When these conditions are met, there is no lateral shifting of the object. When the stage is level, the view is not different from that with the object lying on the regular stage of the microscope. To take the stereo pictures, the stage is inclined about 7° in one direction for the first picture and then 7° in the opposite direction, for the second. No mask is used, since both views fall in the center of the plate, but smaller plates can be used.

In microscopic work, as in ordinary stereoscopic photography, the prints must be reversed in mounting; otherwise a pseudo-stereoscopic effect results. Both plates should have the same exposure and should be developed together so as to yield prints of equal density.

6

Phase and Interference Microscopy

Regardless of the optical perfection of the microscope, ability to see and photograph with it depends on several characteristics of the objects under observation. One of these is color; another is degree of opacity. With objects which are both colorless and transparent, two other factors play definite parts. These are the size (or thickness) of the object and its refractive index (explained on page 191). The latter bears an important relation to the medium in which the object is mounted. If the refractive indices of the two are the same and both object and medium are transparent and colorless, the object is completely invisible; it cannot be seen at all by ordinary means.

Early microscopists soon realized this difficulty and devised means to overcome it. Two techniques provided the answer: (1) to stain the object with a suitable dye, making it visible through its color and nullifying the effect of its refractive index, and (2) to mount the object in a medium with a materially differing refractive index, either a lower or a higher index yielding good differentiation. Suitable mountants were (1) air, with an index lower than any object to be mounted; (2) water, with an index of 1.33, also lower than most mountable objects,* and satisfactory for temporary mounts but not for permanent slides; (3) glycerol and various mixtures and natural resins, ranging from about 1.45 to 1.65; and finally (4) artificial realgar with a high index of 2.30.

It is possible that interest in early days in diatom resolution was responsible for much of the progress made in microscopy, in both theory and practice. Diatoms are extremely minute one-celled plants which secrete an external shell (the frustule) of silica. This shell is transparent and is marked with exceedingly fine detail, of great inter-

* The mineral cryolite is almost identical with water in refractive index, i.e., 1.343.

est to earlier microscopists. Efforts to bring this detail into high visibility — the problem of diatom resolution — produced (besides the development of mediums already mentioned) many advances, such as oblique lighting, the increase in resolving power with increased apertures, the development of apochromatic objectives, Abbe's explanation of image formation through dispersion and interference, and the application of polarized light to the microscope.

Following these, for many years further advance in microscopy was hardly discernible, apart from improvements in microscope designs and auxiliary equipment. But all the time, application of the microscope in scientific and commercial fields was expanding, taking the interest away from the purely amateur field. Thus it was inevitable that scientific research, combined with mechanical ingenuity, should explore new fields in theory and applied optics and that resulting discoveries should be shortly adapted for practical use.

Recent years have therefore given us many new methods, among them phase and interference microscopy. Although new, both of these are built upon foundations laid down long ago, i.e., the part played by refractive-index differentiation, Abbe's theory of image formation, and the principles underlying polarization interference.

While phase and interference microscopy are relatively new and the equipment required is expensive (as compared with the good old days), they have advanced to an important position, especially in biological research. The principles involved in the design of phase-microscopical equipment are often presented in complex mathematical formulae, creating an impression that phase microscopy is of value only to those capable of understanding how it operates. Of course it is true that the more one knows about underlying principles, the better the results one can obtain from the equipment. Yet, fortunately, the equipment itself is just as simple to use as ordinary microscopes. A strictly nontechnical explanation of phase microscopy is much needed for the lay mind and can help pave the way to a complete understanding of the subject.

Phase Microscopy

Let us start with the part refractive index plays in image formation and go back to the problems confronting early microscopists with their diatom resolution. What were the conditions to be met? Diatom shells are composed of silica in the form of opal, with a re-

fractive index of 1.44. Because of their minute size and transparency and the extremely minute surface markings on them, mounting in Canada balsam, with an index of 1.53 does not produce much contrast. Hence a mounting medium of higher index was desirable. This was found in styrax, a natural resin with an index of 1.62. When markings on the diatom are not too fine, styrax is ideal, as is evident from Plate VII, Chapter 10, a micrograph at 6000 \times of a portion of the diatom *Surirella gemma* mounted in it. In this case the index differential is .18. But even styrax is inadequate for the minute diatom *Amphipleura pellucida*, with its dots around 100,000 to the inch. This diatom, mounted in realgar with an index of 2.30 (a differential of .86), is shown in Plate VI, at a magnification of 4500 \times .

Even with this high differential the markings cannot be seen with central illumination. We must resort to another expedient, extreme oblique illumination from a 1.35-1.40 condenser, oiled for full aperture. The decentering of the condenser is apparent in the non-uniform side lighting. This side lighting partakes of two other types of lighting, dark field and Rheinberg illumination (see pages 233-236), as well as what is sometimes referred to as conical illumination, the essential difference being that these latter employ light coming from a full 360° cone, instead of a relatively narrow segment.

It may assist in visualizing some of the factors entering into phase microscopy if we follow through the problem from another angle. Suppose we mount a slide of optical glass having a refractive index of 1.530 in balsam with an identical index of 1.530. As has already been pointed out, the glass particles will be completely invisible and no known technique can reveal them. Even polarized light does not help here (as it would in some cases), for glass is amorphous. If, however, the glass particles possess an index of 1.540 there will be an index differential of .01 on which we can work. Under normal critical light nothing is seen; but by cutting down the cone of light from the condenser to a fraction of that accepted by the objective (creating in effect almost a point source of light) we find that the glass particles function as weak positive lenses. They tend to concentrate the light as the focus of the object is raised, thus delineating the borders by a white line *within the particle* (i.e., the Becke effect; see page 193). Conversely, as the focus is lowered, the white line moves out of the particles into the medium. Thus, by defocussing either above or below the correct focus, there are two possibilities of

seeing the particles. The result is far from satisfactory, however, as is evident from Figure 164. What further steps can be taken with standard optics?

One is to decenter the light, as was done in taking the micrograph of *Amphibleura*. The improvement is obvious, the glass particles showing up in a spectacular manner, as is apparent in Figure 165.

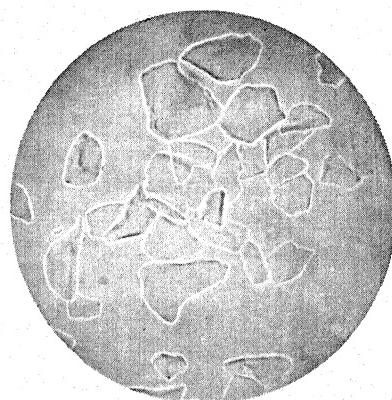


FIG. 164. GLASS (100-MESH) WITH A REFRACTIVE INDEX OF 1.54, IN BALSAM. MAGNIFICATION 40 \times

The condenser aperture has been materially reduced and the focus on the particles has been altered to produce the delineation with a white border (Becke effect) on the outside. The particles would otherwise be practically invisible.

tion with a dark stop not quite large enough to cover the full aperture of the condenser, and closing the substage diaphragm until only a narrow annulus (ring) remains, we have accomplished material improvement over the results achieved by use of a narrow cone of light, as is evident on comparing Figure 168 with Figure 164.

In doing this we have started to utilize some of the principles of the phase microscope, and its use of phase differentiation or phase contrast.

At first thought the question might be raised, "If we can accomplish results such as shown in Figures 166 and 168, why the need for phase

* By means of oblique illumination, or preferably a half-moon diaphragm, the index of particles can be determined accurately to about 5 in the fourth decimal place. See the author's book, *Practical Refractometry*, published by the R. P. Cargille Laboratories, New York City.

Dark field lighting, Figure 167, while providing a brilliant outline of the particles, tells us nothing about their nature. But we are not without further recourse. By blocking off the central illumina-

tion with a dark stop not quite large enough to cover the full aperture of the condenser, and closing the substage diaphragm until only a narrow annulus (ring) remains, we have accomplished material improvement over the results achieved by use of a narrow cone of light, as is evident on comparing Figure 168 with Figure 164.

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microscopes?" There are several answers to this, justifying the need for phase microscopy.

In the first place, the particles at which we have been looking, 100-mesh glass, are very large in comparison with those requiring phase differentiation, 150 microns as against only a few microns. (Thickness of the particles has an important bearing on the degree of differ-



FIG. 165. GLASS (100-MESH) WITH A REFRACTIVE INDEX OF 1.54, IN BALSAM. MAGNIFICATION 40 \times

This is the same field as in Figure 164, illuminated by extremely oblique lighting. The picture is good because the particles are large and produce adequate retardation and dispersion.

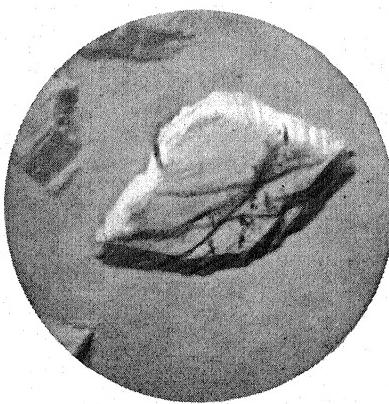


FIG. 166. TREMOLITE (100-MESH) WITH A MEAN REFRACTIVE INDEX OF 1.61, IN BALSAM (R.F. 1.53). MAGNIFICATION 200 \times , UNDER HALF-MOON ILLUMINATION

This is the effect by means of which the refractive index of a material can be determined. The light appears to be concentrated on the side from which it comes—in this case, from above—when the index of the object is higher. It appears to be concentrated on the opposite side when the index of the object is lower. Thus very accurate determinations can be made.

entiation possible, since it, as well as refractive index, determines the resulting phase.)

Then again, it is desirable to discern detail within the confines of a single cell—chromatic material, mitochondria, cytoplasmic structure, inclusion bodies, etc.—all too fine to be seen by the methods so far discussed. One can look in vain in any of the micrographs shown for evidence of fine particles. They are there, nevertheless, in abundance. This can be demonstrated by increasing the differential

between the indices of the medium and the glass particles. In Figure 169 we have 100-mesh glass particles with an index of 1.58 in a medium of 1.65, a spread of .07 index. Here the presence of minute particles can be clearly seen by simply employing a slightly reduced condenser aperture. The reason for the large particles being seen with an index differential of .01 while the minute ones are not is due to lack of sufficient phase difference in the latter. As the phase differential between the object and the medium is decreased, the minimum

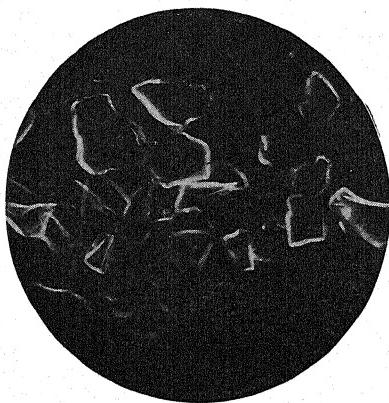


FIG. 167. GLASS (100-MESH) WITH A REFRACTIVE INDEX OF 1.54, IN BALSAM.

Shown by dark field illumination which only outlines the particles and gives no indication of their nature. Magnification 40x



FIG. 168. GLASS (100-MESH) WITH A REFRACTIVE INDEX OF 1.54, IN BALSAM. MAGNIFICATION 40x

The illumination is by means of conical light through a narrow annulus underneath the condenser, with axial light. The cone was very slightly off-center. This method yields results which, while not as spectacular as those of extreme oblique illumination, are as good as can be secured with axial light and without recourse to phase microscopy.

size of particles which can be just discerned decreases accordingly until the limit is reached where no particles of any size can be seen. For this reason, as the limit is approached, it becomes imperative to supply the necessary phase difference by other optical means.

Still another condition exists in the study of biological tissues. It is often desirable to observe them in the living state, or at least unacted upon by fluids which might alter their appearance from that of the living state. This circumscribes the medium in which they must be

examined, which is usually nearly identical with that of the object being studied. Hence the justification for phase microscopy for this type of work.

Before discussing the different variations in optical design by which phase microscopy is accomplished, one must understand what is meant by the term "phase." According to the wave theory of light, light must be considered as an indefinite number of rays proceeding from a single source and vibrating in waves at right angles to the direction of propagation. All rays from a single source are said to be coherent (see page 36) that is, waves of light of any given wave length all start out vibrating in unison: they are "in phase." This is visualized diagrammatically in Figure 170A. As long as the rays are traveling in a medium of uniform density (air, glass, liquid, etc.), they remain in phase; but as they pass into another medium of greater density (i.e., of higher refractive index) they do not travel as rapidly and are slowed down (retarded) while traveling in the denser material. Upon leaving it and passing into the former medium, the rays resume their original speed but are no longer in step with such rays as did not pass through the denser medium: they are now "out of phase." This condition is illustrated in Figure 170B.

If the retardation is exactly equal to one full wave length (or any whole multiple thereof) the waves will still be in phase. But when the retardation amounts to one-half a wave length — so that when the unretarded wave is in its positive phase the retarded wave is in its negative or reversed phase — they neutralize each other and darkness results.* This condition is illustrated in Figure 170C.

* An analogy is offered by two dry-cell batteries, each having a potential of $1\frac{1}{2}$ volts. Connected together with the current flowing in the same direction, the batteries supply a total voltage of 3, but connected in the opposite direction, each bucks the other and no current flows.

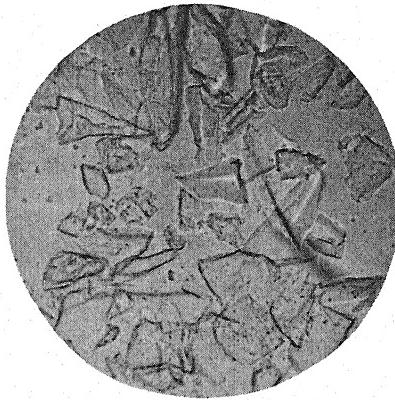


FIG. 169. GLASS (100-MESH) WITH A REFRACTIVE INDEX OF 1.58, IN A MEDIUM OF R.F. 1.65, WITH AXIAL ILLUMINATION. MAGNIFICATION 40 \times

When the condenser aperture is only slightly reduced, the increased differential in refractive index brings out the extremely fine particles

of greater density (i.e., of higher refractive index) they do not travel as rapidly and are slowed down (retarded) while traveling in the denser material. Upon leaving it and passing into the former medium, the rays resume their original speed but are no longer in step with such rays as did not pass through the denser medium: they are now "out of phase." This condition is illustrated in Figure 170B.

Still another effect on light can result from passing through an intervening medium: the amplitude of the waves, which represents the brightness of the light, can be reduced. Such an effect is permanent;

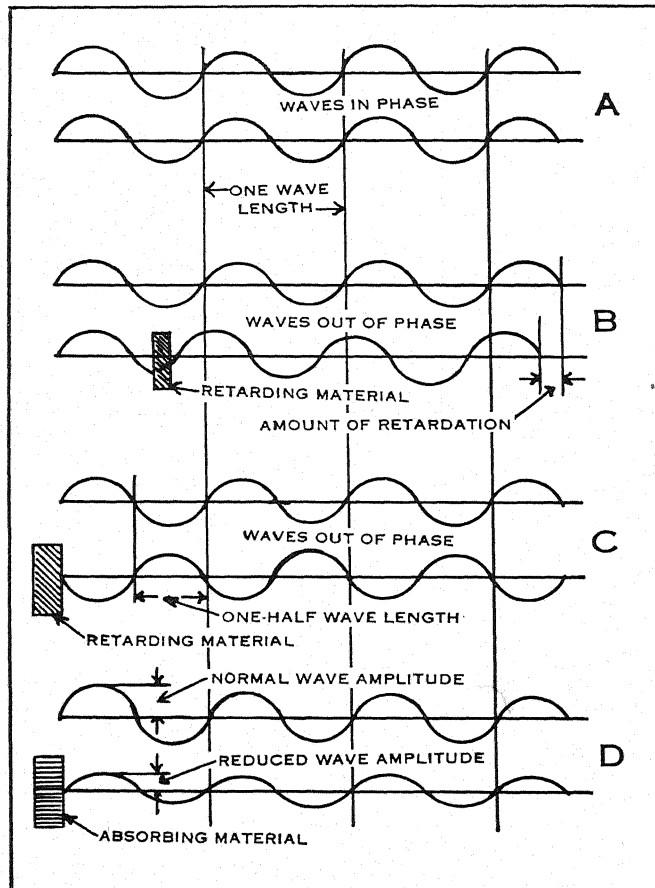


FIG. 170. Schematic Drawing of Wave Motion: A. Waves in phase. B. Waves slightly out of phase. C. Waves in opposite phase. D. Reduction of amplitude by passage through light-absorbing material.

the light does not increase again in brightness when it has once been reduced. The condition is shown in Figure 170D. An application of this principle is the use of smoked glass to observe an eclipse of the sun, or the use of tinted glasses to offset the brilliance of summer sun-

light. Both of these effects on light waves are made use of in various types of phase optics.

While the function of phase differentiation in the formation of microscopic images was known for many years, this knowledge was not applied until Zernike in 1935 proposed a practical way to accentuate contrast by the incorporation of auxiliary phase plates in the optical system of the microscope. The first microscopes to incorporate Zernike's idea were made by Zeiss some few years later. Other manufacturers soon followed suit, either following Zernike or utilizing modifications and other principles of attaining equivalent or variable results.

Basically, two additions to the optical train are necessary to adapt a standard microscope for phase microscopy — a ring diaphragm under the condenser and a corresponding phase plate placed in the rear focal plane of the objective. The former corresponds to the annulus illumination by means of which Figure 168 was taken. In appearance it is like the diaphragm shown in Figure 171, and such a diaphragm could be used with any substage condenser. The only requirement would be that the diameter of the central portion must be less than the diameter of the cone of light accepted by the objective (i.e., its N.A., numerical aperture); otherwise it would cut out all direct light and dark field would result. It will be obvious from this that each objective with differing N.A. will require a different diameter for the annulus, a condition which can be met in either of two ways, (1) supplying a complete set of ring diaphragms, one for each objective, which can be mounted in the ring holder underneath the condenser, or (2) supplying a special condenser equipped with a rotating mount holding the necessary diaphragms, which can be rotated into position for the corresponding objective.

With the proper diaphragm in position beneath the condenser and the latter focussed on the plane of the object, the objective (also focussed on the plane of the object) picks up the hollow cone of light from the diaphragm and images it as an identical ring in its rear focal plane. This being the case, it will be apparent that with a plate mounted in the rear focal plane of the objective having an opaque ring in it exactly corresponding to the image of the condenser annulus, all light would be blocked off and none would reach the eyepiece if no object were on the stage. But with an object on the stage, differing ever so slightly from the medium in which it was mounted, the light is broken up into two factors, one portion being the undevi-

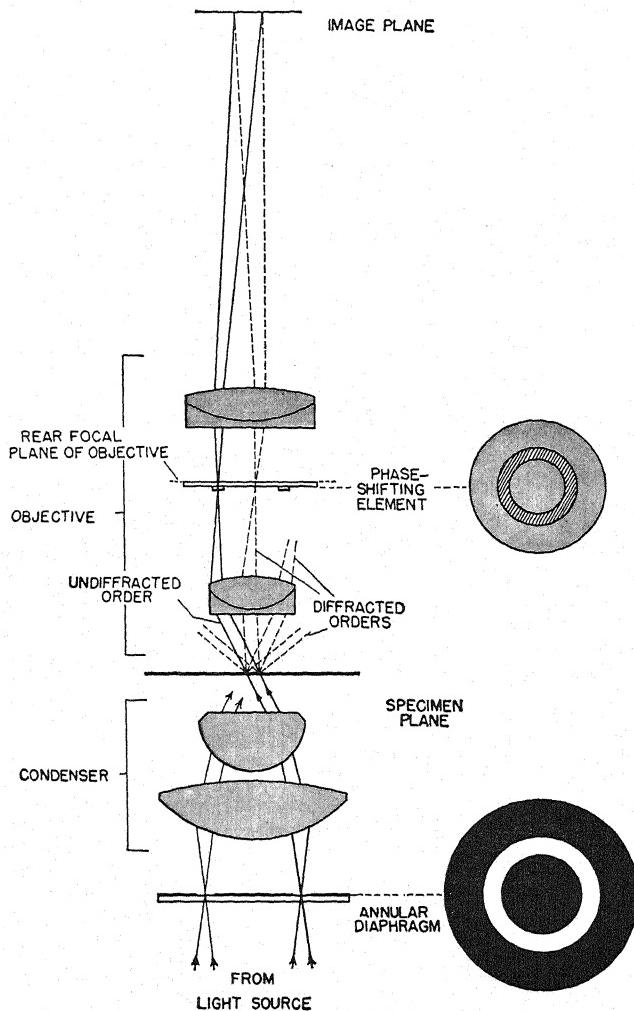


FIG. 171. The Path of Light Rays in a Phase-Contrast Microscope. (Courtesy, Bausch & Lomb Optical Company.)

ated light imaged on the rear focal plane of the objective and the other diffracted and retarded * by the object and spread over the entire area of the rear focal plane. Hence, with an opaque annulus in the objective, dark field illumination would result, the object appearing

* That is, if the object has a refractive index *higher* than the medium. If lower, the retardation occurs in the medium.

bright on a dark background. With low contrast in the object and with the opaque annulus removed from the objective, the undeviated light constitutes such a large percentage of the whole that it floods out the diffracted rays until they cannot be seen at all, or at least but dimly. The fact that the diffracted rays, as well as the undeviated ones, come to a focus at the rear focal plane of the objective allows the annulus plate located in this position to modify the resulting image in several ways. Ordinarily there is no advantage in securing dark field with an opaque annulus, but by means of a very thin metal

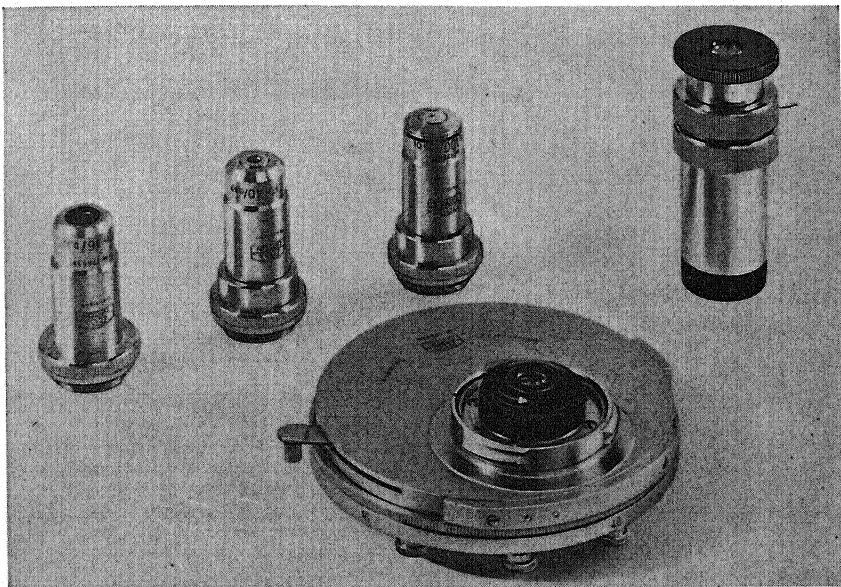


FIG. 172. Carl Zeiss Phase-Contrast Accessories: A Rotating Condenser, Special Objectives, and a Centering Telescope

deposit forming the annulus it is possible to cut down the amount of undiffracted light to any degree desired—in other words, to reduce the amplitude of the waves, as in Figure 171, to any opacity which will provide the amount of light transmission from zero to a maximum.

Then on the other areas inside and outside of the annulus, a layer of retarding material can be placed, the phase retardation so provided being added to that produced by the object itself, thus intensifying the contrast. In this way the increased contrast, in combination with a suitable reduction of the intensity of the undeviated light, can give

an effect similar to that seen in the microscope with a stained or naturally contrasty specimen. This is known as dark contrast and is the basis of the phase microscope as proposed by Zernike. The phase microscopes supplied by Zeiss and by Bausch & Lomb use this system.

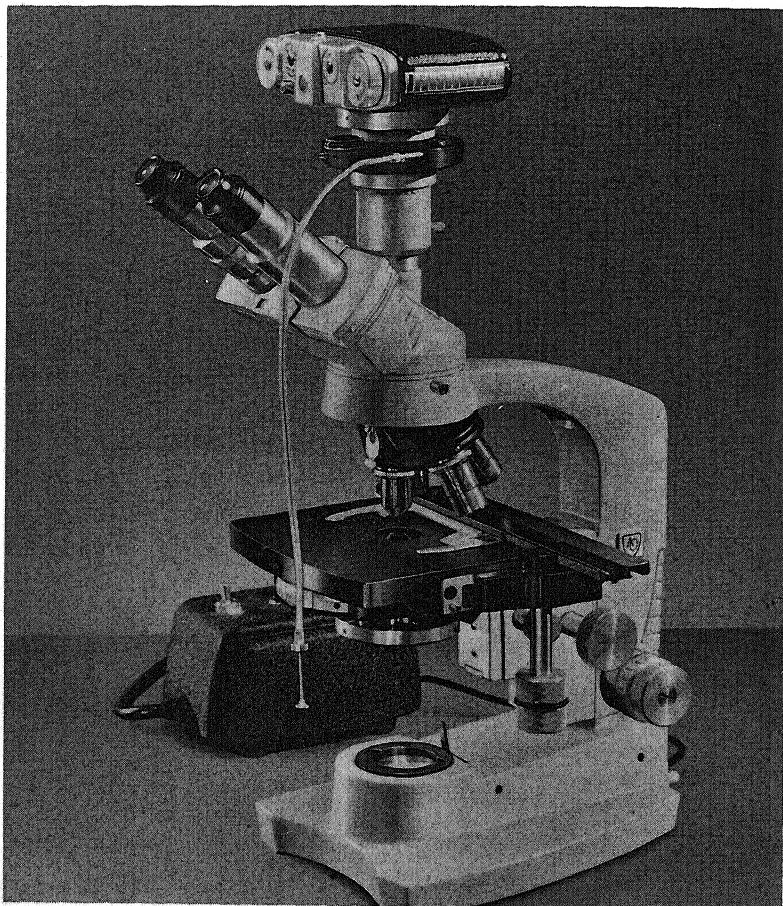
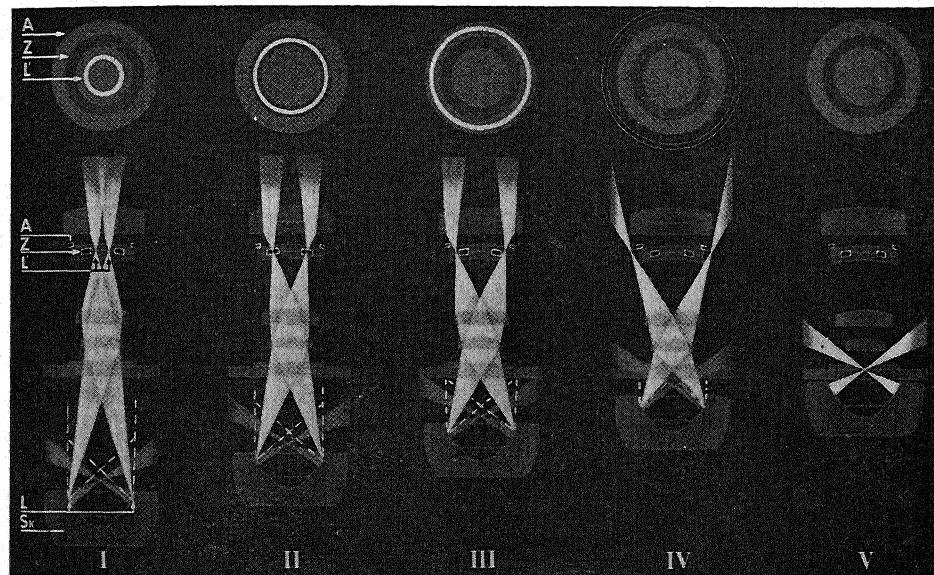


FIG. 173. American Optical Company Phase Microscope, Model L6TU-P4

By varying the combinations of phase-retarding material and light-absorbing material on both the annulus and surrounding areas of the phase plate, almost any combination of illumination of the object and the field can be secured. The American Optical Company furnish three sets of objectives to take advantage of variations possible. Thus

one can have bright contrast, dark contrast, or intermediate, as desired. For such as require but one set of objectives, the dark contrast is usually the most useful, since it gives an approximation of results obtained by staining, or from contrasty objects. In some cases better results can be obtained with bright field or with what is known as "B contrast," which is intermediate between the two extremes.

The American Optical Company supply their microscope outfit complete, equipped with objectives and eyepieces, as ordered. Other



1. The mirror body (*Sk*), when in its lowest position, produces a small diameter light ring (*L*) which is imaged in position (*L'*) inside the Zernike phase ring (*Z*). Bright field observation.

2. Upon raising the mirror body (*Sk*), the image (*L'*) of the light ring becomes larger in diameter until it is completely covered by the dark appearing phase ring (*Z*). Setting II, namely, phase contrast after Zernike, has now been reached.

3. When the mirror body (*Sk*) is raised further, the diameter of the illuminated ring becomes still larger until it is outside of the phase ring. This position III produces very contrasty images in bright field.

4. Raising condenser still further allows the light ring image to disappear completely behind the edge (*A*) of the aperture diaphragm. In position IV, therefore, light ring (*L*) is the source of light producing a dark field which when compared with standard dark field illumination gives particularly clear structural details.

5. In position V the converging illuminating rays become effective; therefore, we have reached standard dark field illumination.

FIG. 174. THE OPTICAL PRINCIPLE OF THE LEITZ VARIABLE PHASE-CONTRAST MICROSCOPE

manufacturers (Bausch & Lomb, Leitz, Zeiss) supply either complete outfits or the phase optics only for use with standard microscopes.

The phase outfit supplied by Zeiss (Western Zone) is shown in Figure 172. The American Optical Company's Model L6TU-P4 Microscope, equipped with the set of phase optics is shown in Figure 173. This microscope can, of course, be used as a regular microscope by equipping it with standard objectives.

The Leitz phase microscope employs a radically different type of illumination. It substitutes for the ring annulus in the substage con-



FIG. 175. Leitz Phase-Contrast Equipment

denser a Heine mirror condenser which functions as a ring illuminator. The Zernike phase plate, as used in other phase systems, is used in the various objectives. An advantage gained by this type of condenser is that the ring illumination can be varied continuously by racking it up and down, thus obtaining any type of lighting, from ordinary bright field through different modifications of phase to ordinary dark field. Figure 174 shows how this is secured by varying the position of the condenser. Position III is the position where equivalent Zernike phase is secured. Figure 175 shows the phase

equipment supplied by Leitz, which can be adapted to any research stand.

The statement was made (page 274) that phase equipment is just as simple to use as ordinary microscopes, but this requires qualifying. It is correct only on the basis that one knows how to get out of an ordinary microscope all that it is capable of giving, involving ideal lighting conditions, the proper use of filters, the ideal combinations of objectives and eyepieces to employ to accomplish a desired result, and the satisfying of all conditions contributing to an ideal result. The number of would-be microscopists measuring up to all these requirements is small indeed; such, however, as do will find no difficulty in the operation of a phase microscope.

Two factors, in particular, contribute to perfect performance. These are (1) the employment of critical illumination, just as with the ordinary microscope, properly controlled for intensity, and (2) perfect centering of the phase components. It is very important that the annulus of the condenser and that in the objective be perfectly aligned in the optic axis. Mechanical imperfections in the optical equipment require that centering adjustment be provided to meet this condition. Such centering can be accomplished by means of individually centering objectives or within the rotating member holding the condenser annuli. To perform the centering operation it is necessary to observe it at the back lens of the objective while it is being done. For this purpose a viewing telescope eyepiece (also known as an auxiliary microscope) is provided for insertion in the tube in place of a standard eyepiece. The objective annulus is slightly wider than that of the condenser; hence the centering is easily accomplished by whatever arrangement is provided. After this is done the telescope viewer is removed and replaced by the eyepiece. Unless permanent centering of all objectives with their corresponding condenser annulus is provided, it is necessary to check the centering each time a change in objectives is made.

One must learn how to obtain ideal visual images before attempting phase photomicrography. It is hardly necessary to state that while visual use of phase microscopy is important in early stages of research work, photomicrographs will usually represent the end product of the work. Photographs are not only important for progress reports, but finally for conveying the results of investigations to others. For this reason it has been deemed advisable to include fairly complete information on the theory, equipment and practice of phase micro-

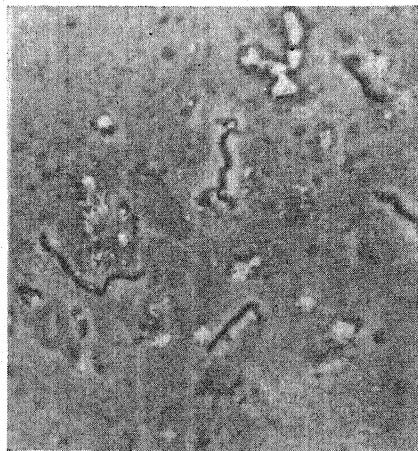


FIG. 176. *Leptospira icterohaemorrhagiae*. A faded specimen taken by phase contrast

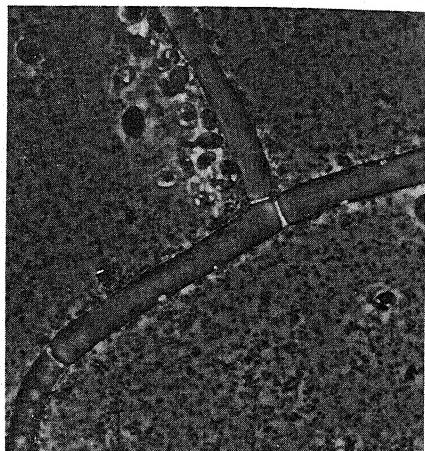


FIG. 177. Yeast Cells, Mold Mycelium, and Bacteria, in Culture. Magnification 258 \times , by phase contrast. (Courtesy of Bausch & Lomb Optical Company.)



FIG. 178. Blood Vessel of Rat. An unstained section mounted in balsam. Magnification 135 \times

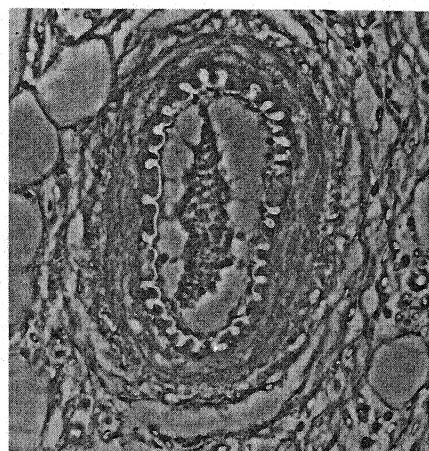


FIG. 179. The Same Section as in Figure 178, Photographed with Phase Contrast by Bausch & Lomb Equipment

copy in a book primarily intended for photomicrography. Assuming good photomicrographic technique with the ordinary microscope and a thorough knowledge of phase microscopy, no difficulty will be experienced in securing good pictures of objects and structures to be seen only by means of the phase microscope.

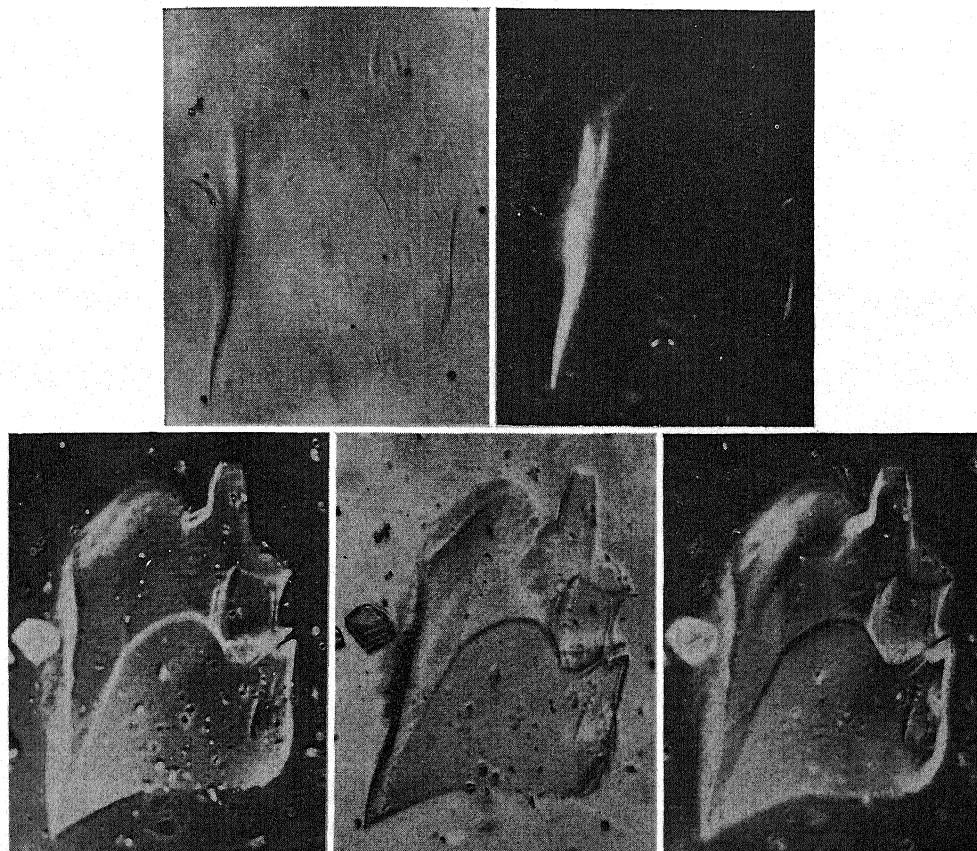


FIG. 180. A Glass Particle Mounted in a Medium with a Refractive Index Near That of the Glass. Taken with the American Optical Company's Phase-Contrast Microscope, these pictures show the specimen in ordinary bright field, in dark field, and in three types of contrast attainable with the American Optical Company systems

Figures 176 to 184 show what can be accomplished with phase microscopy. They represent results obtained with instruments provided by the various manufacturers of phase equipment.

Interference Microscopy

Interference microscopy represents a further advance on phase-contrast microscopy. It is a relatively recent development in micro-

scopical science, and hence to a certain extent may be said to be still in its infancy.

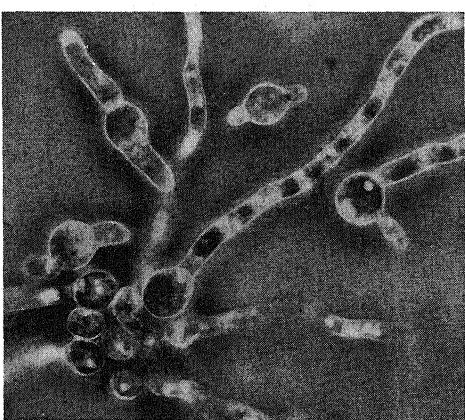


FIG. 181. Germinating Spores of *Penicillium glaucum* under One System of Phase Contrast. Magnification 1000 \times ; with the Leitz Phase Condenser. (Courtesy, E. Leitz, Inc.)

Although interference microscopes can be employed for much the same purposes as phase microscopes, interference microscopy, as employed in cytological research work, finds its greatest value in the measurement of the optical thicknesses and associated dry mass weights of minute objects such as individual cells and the various components thereof. The degree of optical thickness measurements which can be achieved by this means extends to an optimum accuracy of 1/300 of a wave length of light.

Two different systems of optical means for securing interference, both originating in England, have been developed and incorporated in microscopes that at present writing are the only commercial outfits available on the American market. Since interference effects are a natural consequence of this form of microscopy, these systems utilize polarization phenomena in their design and to this end are equipped with polarizers and analyzers.

In addition to these, a third radical design of instrument, known as a variable phase-contrast microscope, represents a further extension of phase microscopy; since the phase contrast is provided within the instrument itself by polarizing elements and quartz phase plates, it does not require special annuli objectives but uses standard types. The

Like phase contrast, interference microscopy yields contrasty images of objects that would be poorly delineated under ordinary light microscopes. It does this by means of a split-beam interferometer system incorporated in the optical system of the microscope itself, rather than by diffraction effects resulting from the refractive index and thickness of the object, accentuated by optically introduced additive and subtractive phase, as described in the preceding section.

chief characteristic of this system is that absorption in the phase plate is continuously variable throughout the whole range of possible types of contrasts. The instrument is shown in Figures 185A and 185B, as employed for visual work.*

When photomicrographs are required, the camera is fitted on top of the vertical tube and the light switched from the binocular eyepiece to the vertical. Phase contrast with variable amplitude (and if desired, variable phase) is possible at all magnifications. Both polarized and nonpolarized light may be employed; hence a combination of both phase and interference effects can be achieved.

The unique feature in this design lies in the method of securing ample tube length for the introduction of the polarizing elements — polarizer, analyzer, quartz phase plates, and possibly other accessories necessary to effect amplitude variation with equal efficiency at all powers and at the same time employ standard objectives. The substage condenser is equipped with an annulus similar to that employed in phase-contrast microscopes.

As can be seen from the schematic of the optical system, Figure 186, adequate tube length is accomplished by the addition of a supplemental horizontal tube having a concave mirror at the outer end. The primary image of the back focal plane of the objective, after deflection by a 45° mirror into the horizontal tube, lies in the plane of the concave mirror. The focal point of the latter is of ample length to re-form an image of the back focal plane of the objective, after a second right-angle deflection into the optic axis of the microscope, at a suitable position to allow the insertion of the necessary accessories. A Bertrand lens can be inserted for focussing the plane of the phase plate when required. A slot is provided in the body

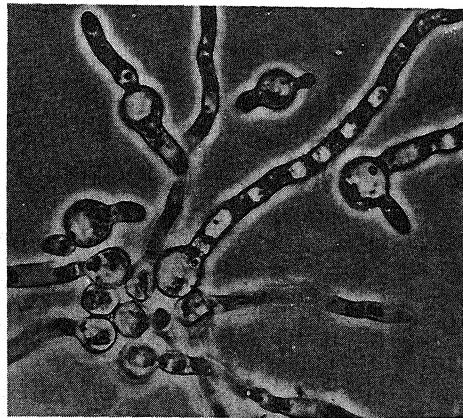


FIG. 182. The Same Specimen as in Figure 181 under Another System of Phase Contrast Using the Leitz Phase Condenser. (Courtesy, E. Leitz, Inc.)

* It is manufactured by Cooke, Troughton & Simms of England. American representatives are The R. Y. Ferner Co., Inc., 110 Pleasant Street, Malden 48, Mass.

tube to enable a compensator to be inserted to effect still further phase variation over the quarter-wave retardation produced by the quartz phase plates.

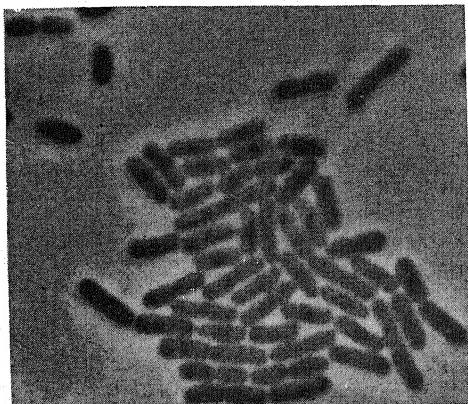


FIG. 183. *Pneumococcus* as It Appears in a Clear Agar Culture. Taken with Leitz Phase Equipment; magnification 3400x. (Courtesy, E. Leitz, Inc.)

ponents, one transmitted and one reflected at the internal first surface of the lower plate and follows a path parallel to the transmitted ray. The transmitted ray passes through the object to the top interference plate where it is then doubly reflected to combine with the other ray which did not pass through the object. The combined rays then enter a spherical glass segment, completely silvered on the spherical surface except for a small clear area in the optical axial center. Here all rays, after a second internal reflection from the partially aluminized bottom surface of the spherical segment, are brought to a focus, imaging the object where it can be picked up by the objective. The latter must be of relatively high power (either 4 mm. .85 N.A. or 1.8 mm. 1.30 N.A.).

It will be evident (since all rays from the condenser pass through the system and while axial rays are undeviated, those at an angle are increasingly separated) that bands of color (Newton's rings) are produced through several orders. Were both interference plates optical flats and perfectly parallel with each other, no means for securing variations in image effects would be available, but the plates are

The same company also manufactures the Dyson Interferometer Microscope. It is shown in Figure 187, and a schematic of the interferometer principle in Figure 188. In this design the interference is produced through the use of two identical optical glass plates, one located below and one above the object plane. Both surfaces of each are partially aluminized to an extent sufficient to divide the light rays from the substage condenser impinging at an angle into two substantially equal com-

slightly wedge shaped (a 5° angle) and means for traversing one across the other then allow for separation of the color bands as desired. With monochromatic light the bands are dark, while objects located within them are bright. The same objects when in the bright portion of the bands are dark. This is illustrated in Figure 189. With this system objects are not surrounded with a bright border so characteristic of ordinary phase-contrast microscopy. The difference can be seen in Figure 190.

The other type of interference microscope which is becoming increasingly popular in America is the Baker type, manufactured by the American Optical Company.

In this instrument the mutually interfering beams are secured by the use of calcite plates, one associated with the substage condenser and located directly above it. A similar plate (of proper diameter) is mounted in front of the objective as an integral part of it. The first splits the light beam into two parallel components, which are recombined by the second, following interference by the object. Two different methods of securing interference are employed — one known as the double-focus system, the other as the shearing system. Each of these requires three different powers of objectives ($10x$, $40x$, and $100x$) to cover the entire range of magnification, with matching condensers for each objective.

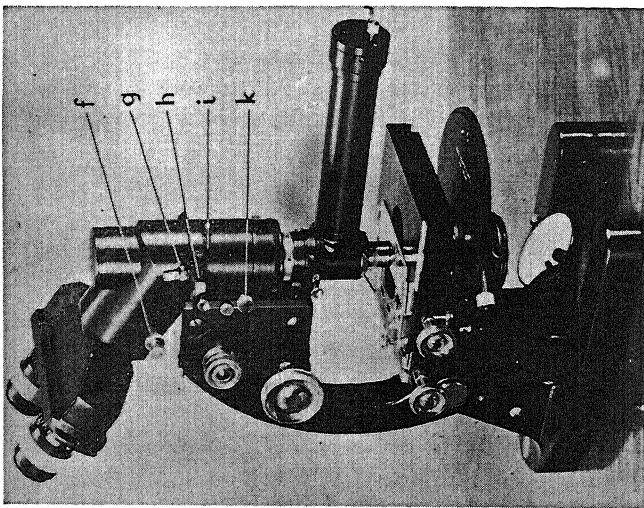
In securing the sharp primary image in each system a secondary focus (known as the reference focus) results. In the double-focus system this secondary focus lies in the optical axis above the primary image, while in the shearing system this reference focus is thrown to one side, although still in the field.

The double-focus system is of value especially in the study of objects of low contrast. To a certain extent it corresponds to phase-contrast microscopy, the image being secured by means of optical



FIG. 184. A 24-Hour Culture of Living Chick-Heart Fibroblasts. Taken with Leitz Phase Equipment; magnification 1450 \times . (Courtesy, E. Leitz, Inc.)

B



A

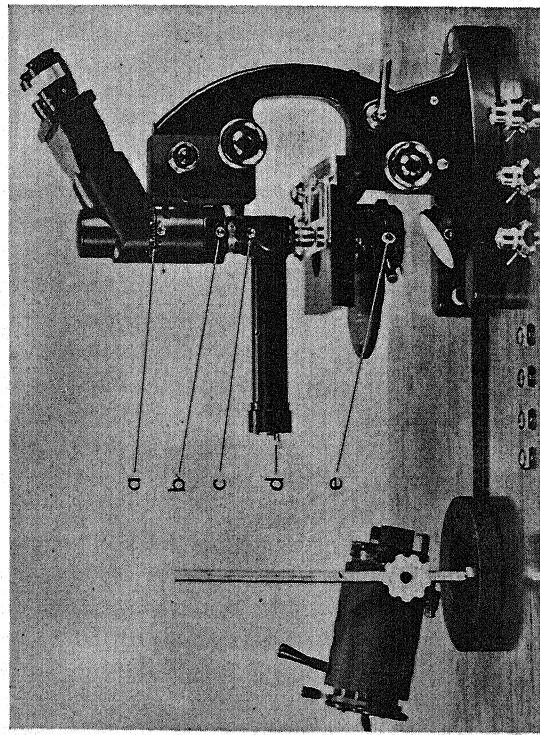


FIG. 185. THE VARIABLE-AMPLITUDE PHASE-CONTRAST MICROSCOPE, IN TWO VIEWS; A, WITH ILLUMINATOR:
a—Amplitude-variation control. *b*—Phase-plate slide. *c*—Polarizer. *d*—Mirror-tilting slide. *e*—Annulus-centering screws. *f*—Bertrand lens control. *g*—Trip lever for photomicrography. *h*—Analyzer slide. *i*—Slot for compensator. *k*—Phase-plate rack adjustments.

interference. With white light, this implies color variation instead of increased light-intensity contrast due to phase variations.

The shearing system is primarily intended for the measurement of

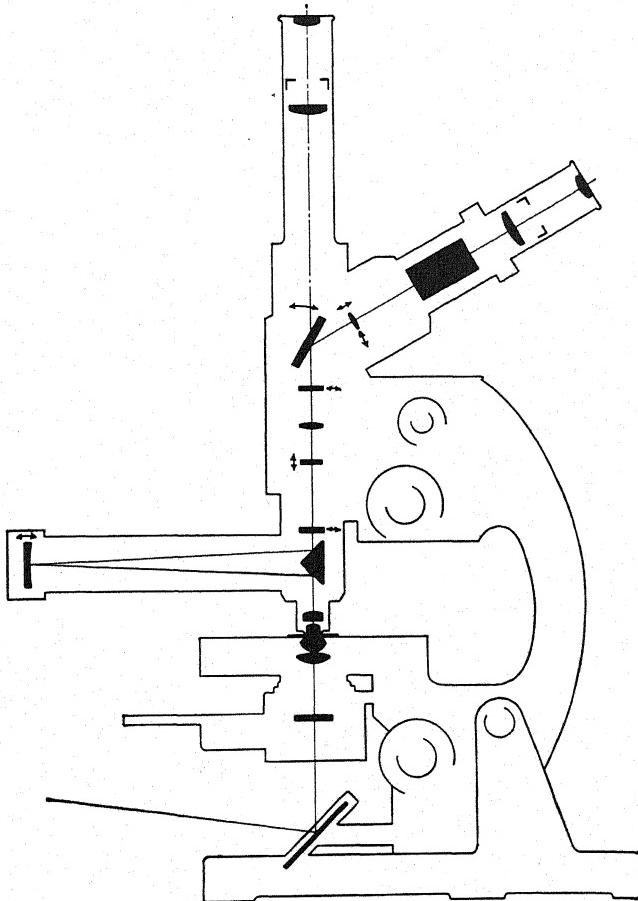


FIG. 186. A Schematic Diagram of the Optical System in the Cooke, Troughton & Simms Variable-Amplitude Phase-Contrast Microscope

optical thickness and the corresponding dry mass weight of individual cells and their various components.

The polarizing elements are Polaroid, and the analyzer is rotatable through 240° registered on an engraved scale graduated in 2° inter-

vals. The polarizer is located below the condenser and can be rotated into a position which provides for ordinary bright field microscopy. A one-quarter-wave plate located below the analyzer can be inserted or withdrawn as desired.

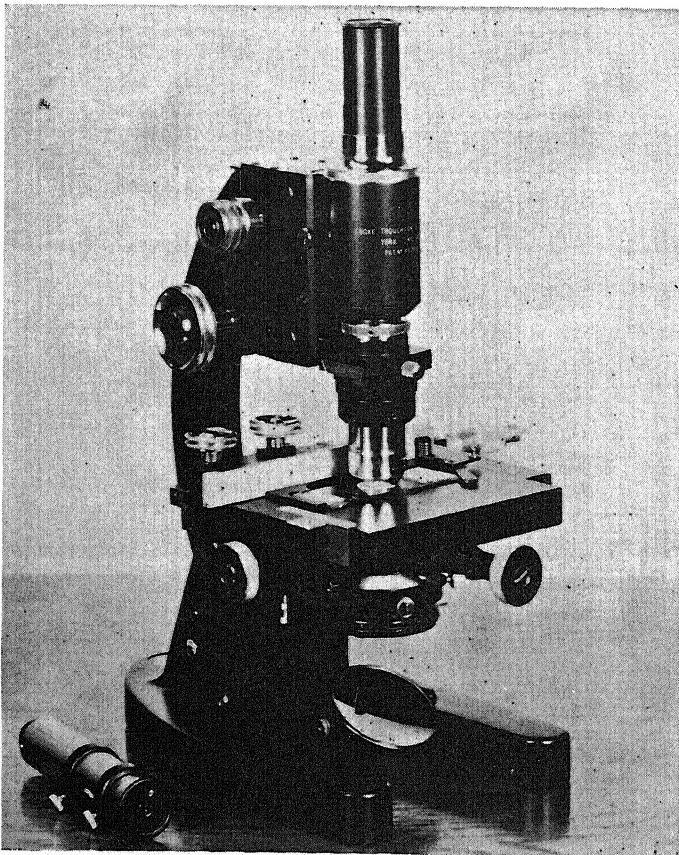


FIG. 187. The Cooke, Troughton & Simms Dyson Interferometer Microscope

In the shearing system, in order to select the proper order of interference color for the field, the condensers, with their associated beam-dividing plates, can be tilted out of axial alignment. For accurate measurements of optical thickness it is necessary to employ monochromatic light through the use of a monochromator or narrow-band

filter. Measurements are made possible through the change of color resulting from altering the interference in the system. Figure 191

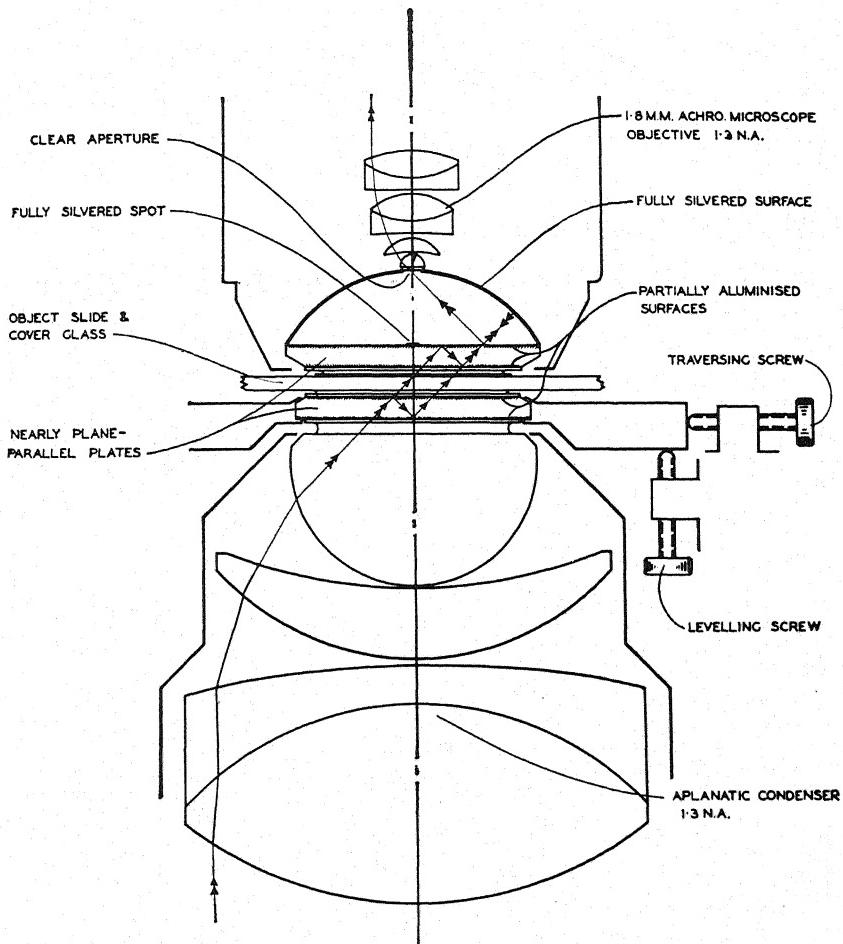


FIG. 188. A Schematic Diagram of the Path of Light Rays in the Dyson Interferometer Microscope

shows the latest design of the A.O.C.-Baker Interference Microscope and accessories.

While cytological research where interference microscopy is essential is still in its infancy, it can be predicted that the future will yield

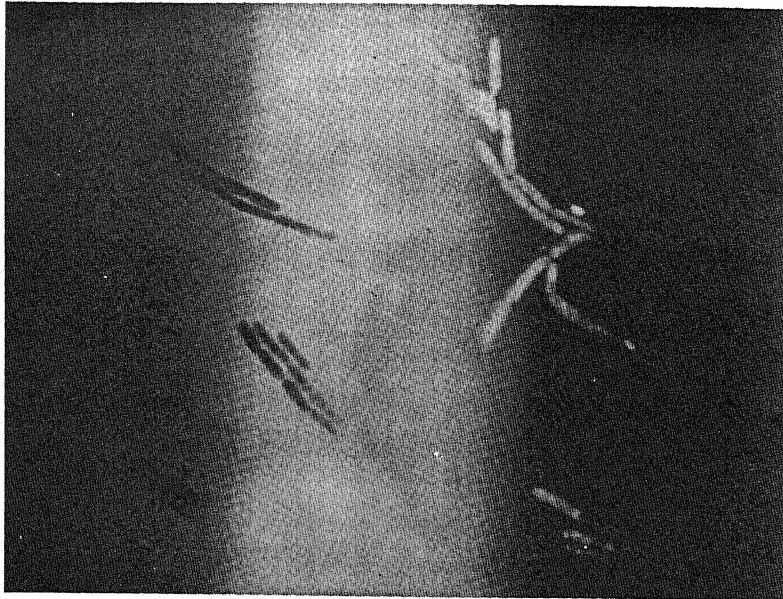


FIG. 189. A Living Preparation of *Bacillus cereus*. Magnification 1200 \times . The contrast varies with the position in the field. Taken with the Dyson interferometer microscope

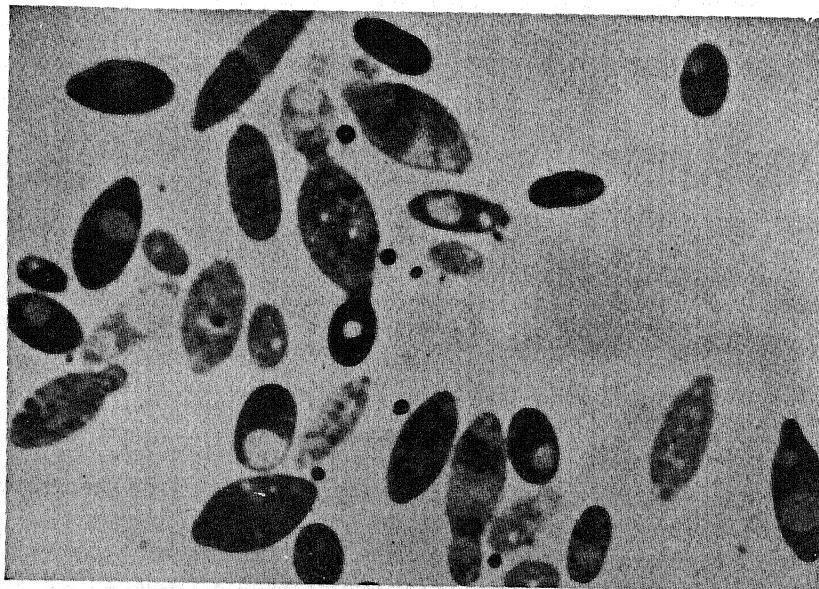


FIG. 190. A Living Preparation of *Kloeckera brevis* Taken by Bright Field Contrast. Magnification 2360 \times . The darker areas have the greater dry mass per unit of area. Taken with the Dyson interferometer microscope

much useful data through the use of these special instruments. Figure 192 illustrates what can be done at 3000 \times .

It is hardly necessary to add that interference microscopes call for much ingenuity from a theoretical design standpoint, combined with

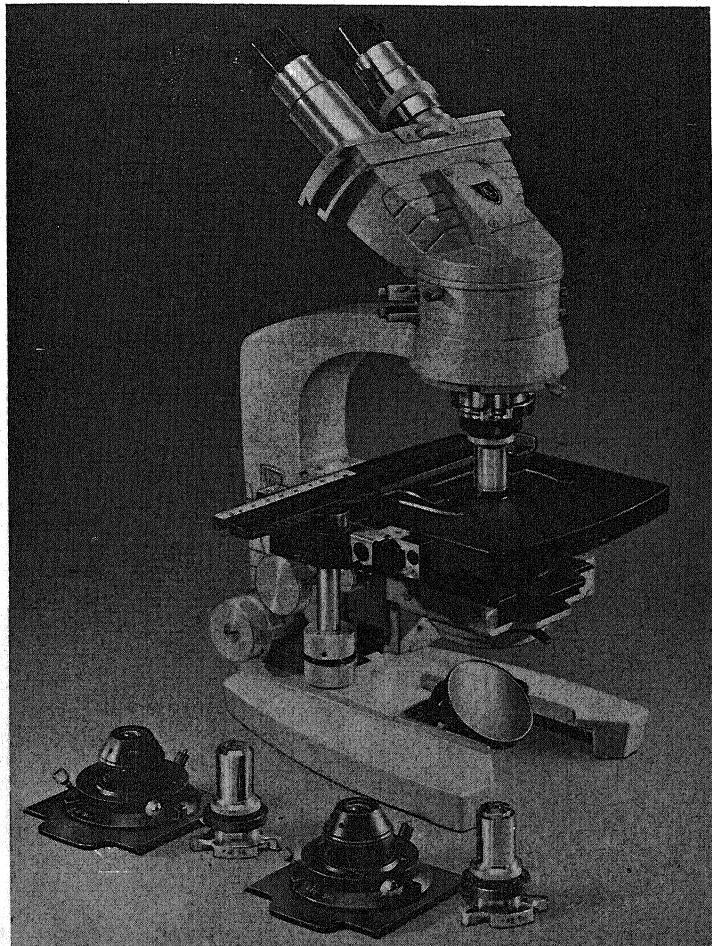


FIG. 191. American Optical Company Interference Microscope, Model 7BG-QSW

mechanical and optical accuracy of the very highest order. This implies ultimate costs for the complete outfit many times those of ordinary research microscopes.

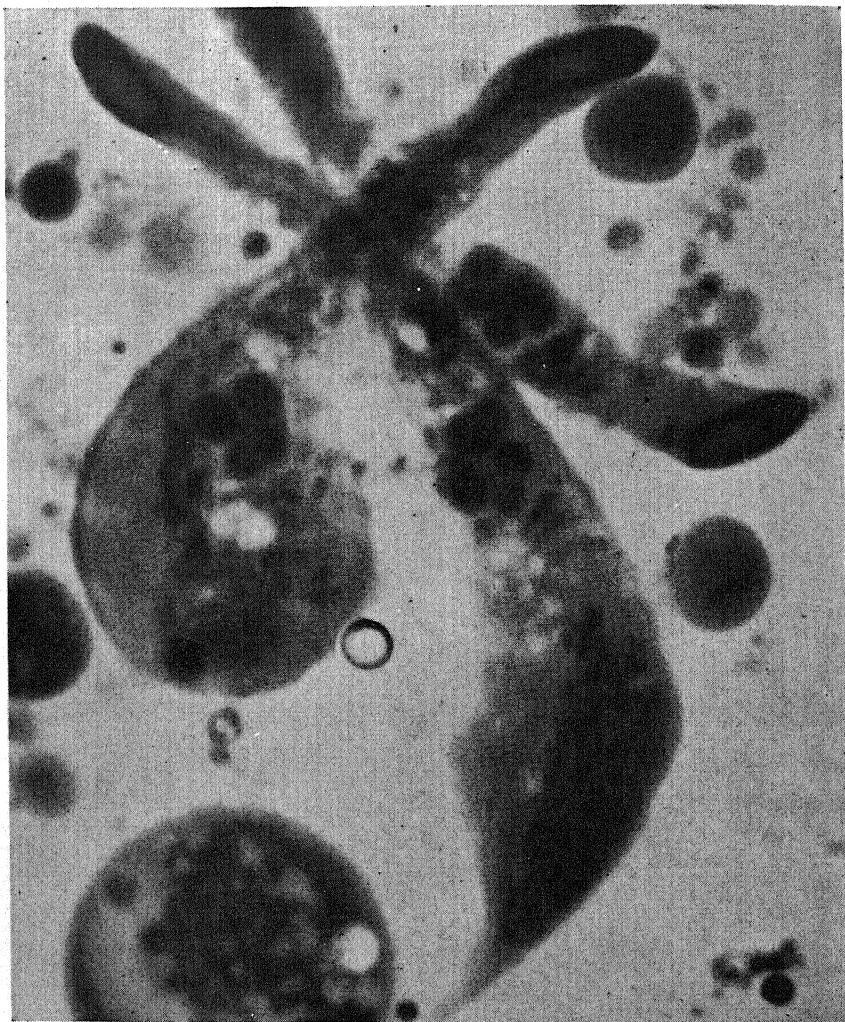


FIG. 192. SPERMATIDS OF THE PULMONATE SNAIL (*Otula lactea*) MAGNIFIED 2970X

This photomicrograph was taken with the American Optical Company Interference Microscope, double-focus system, with a 100 \times objective, a 10 \times Periplan eyepiece, and a green filter, from a smear in saline solution. The contrast has been adjusted to produce the darkest tones in the sperm head. In the spermatids there can be seen the sperm tail, Golgi bodies, mitochondria, etc.

The Electron Microscope

In recent years the electron microscope has made enormous strides in improved design, simplicity of operation, increase in magnification range, and application to wider fields of usefulness, and it is now accepted as an essential instrument in many lines of research. Though many of the original models of the R.C.A. microscope (Figure 193) are still in service, comparison with later models will reveal the extent of the changes in design which have taken place.

The underlying principle of the electron microscope has not been changed, i.e., the utilization of streams of electrons, negatively charged particles of matter, which act as extremely short wavelengths to produce the image. These (cathode rays) are generated through the use of very high voltages (up to 100,000 volts), such as those employed in X-ray work.

There are no lenses of transparent material in the electron microscope, their place being taken by magnetic lenses — electromagnetic coils which cause the cathode rays to follow definite courses, an effect similar to the refractive effect achieved in a light microscope by the use of glass lenses. There must be a minimum of three such magnetic lenses, corresponding to the substage condenser, the objective, and the eyepiece of the light microscope. Since the electron microscope must provide a wide range of magnification and it is also arranged for producing atomic diffraction patterns, additional coils are necessary and the total number of coils usually is five. One of the extra coils is required for diffraction work, the other for projecting the high-magnification range. The image is observed visually on a fluorescent screen (as in a television set) and can be photographed on sensitized film or plates. Figure 194 shows diagrammatically the path of the rays for low and high magnifications and diffraction patterns.

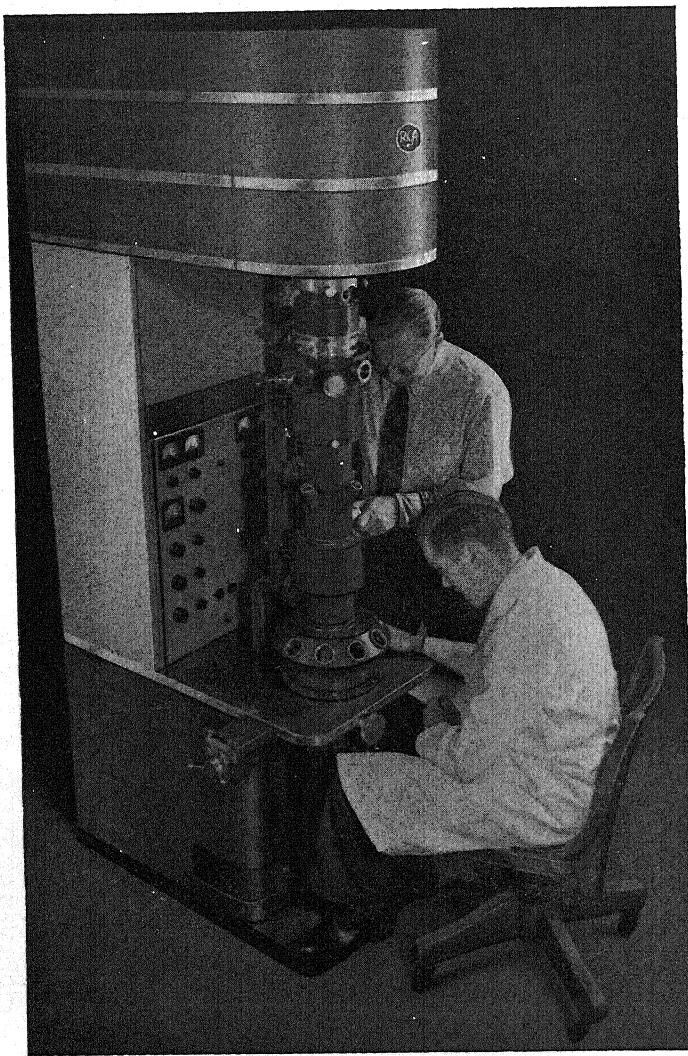
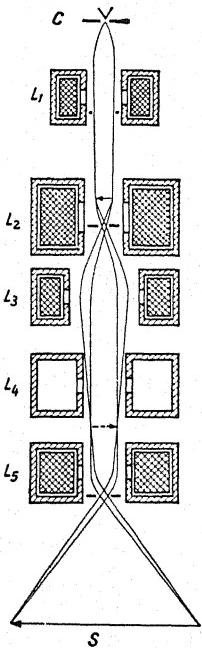


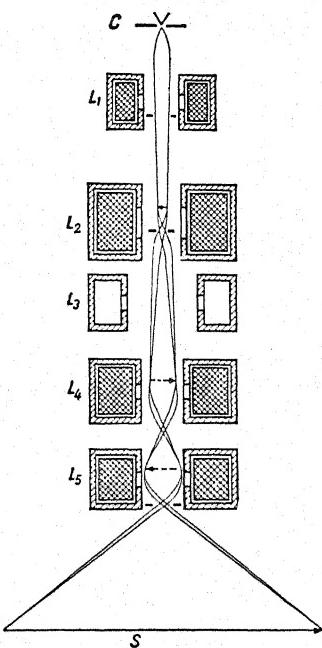
FIG. 193. An R.C.A. Electron Microscope, Early Model

Resolution obtainable with the electron microscope follows much the same laws as apply to the light microscope (see Chapter 1). In the latter the maximum theoretical resolving power depends on the shortest usable violet (or ultra-violet) wavelength combined with the highest possible numerical aperture (N.A.). In the electron

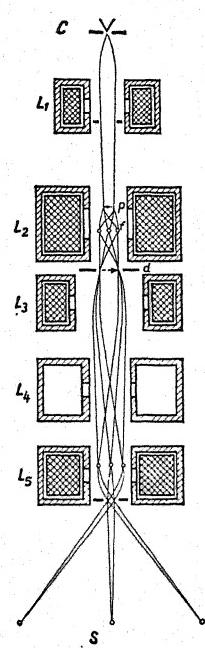
microscope the N.A. cannot be very high, but the extremely short "wavelengths" (produced with the highest voltages available) provide resolution running into hundreds of thousands of times. To obtain the maximum magnification possible it is customary to make



Electron path at low magnification (4,000X), intermediate lens not energized.



Representation of the electron path at high magnification (4,000-60,000X) diffraction lens not energized. L₁ condenser, L₂ objective, L₃ diffraction lens, L₄ intermediate lens, L₅ projector, C electron gun, S fluorescent screen.



The electron path when carrying out diffraction work. On the screen, an image is produced of the focal plane, f, at the image side of the objective lens and not of the object plane, p. An intermediate image of p is formed in the plane d.

FIG. 194. Diagrams of the Paths of Electron "Rays" in an Electron Microscope Under Varying Conditions. (Courtesy, Philips Electronics, Inc.)

positive enlargements from the original photographic negatives. These negatives are so sharp that detail is not lost by enlarging up to several times. From this it is evident that maximum magnification is not possible in the visual images but it is still far beyond that obtainable with the light microscope.

One big advantage the electron microscope possesses over the light microscope, especially in the high range of magnification possible in the latter, is that the depth of focus is much greater. With the high-aperture lenses required for high magnifications in the light microscope, the focal plane has practically zero depth of focus; even a round body only one micron in diameter (e.g., a spherical micrococcus) cannot be in focus for the center and periphery at the same time. In the electron microscope the depth of focus may amount to several microns, and objects of considerable thickness appear much sharper in an electron photograph.

Still another advantage inherent in the electron microscope lies in the fact that the electron image is produced entirely by the difference in degree of absorption of the cathode rays by an object and staining is not required, nor is phase difference an important factor as is the case with a light microscope. On the other hand, while the depth of focus is adequate to allow tissue sections a few microns in thickness to be sharply defined, the absorption is so great that no rays can pass through them. For a long time this constituted a serious drawback to the use of the electron microscope in histological and pathological work, since microtomes capable of cutting sections less than a micron in thickness were not available. This problem has been solved by the development of several types of microtomes capable of cutting sections from $1/10$ to $1/40$ of a micron thick. Such sections can be studied with the electron microscope and much is being learned from them.

Another application of the electron microscope to microscopical problems, i.e., the study of surfaces of opaque objects (e.g., metals) which is accomplished with the light microscope by means of reflected light, has been solved by making replicas of such surfaces on thin films of plastic or synthetic resin. The plastic is dried and stripped off, and yields a negative cast in which all features are shown in reverse. A positive film must be made from this if there is to be a true rendition of the original surface. When such a film is viewed by transmitted light, it is customary to shadow-cast the surface to bring out the highlights and shadows. This is accomplished by exposing the film to metallic vapor emanating from a heated metallic filament, usually chromium (several other metals are also used, among them nickel, platinum, palladium, and manganese). The amount of shadowing, the angle at which the shadows are cast, and the preferred metal to employ are determined by the roughness of the surface and

the length of the shadows which will give the best three-dimension effect in the picture.

Beautifully sharp pictures of metal surfaces at very high magnifications have been taken by the replica method. While replicas portray the true nature of the surfaces shown and provide ease of interpretation of them, it must be admitted that very little additional knowledge of metal structures has been derived from them, beyond that revealed by high-quality micrographs taken with the light microscope. For instance, little more is learned regarding the nature of

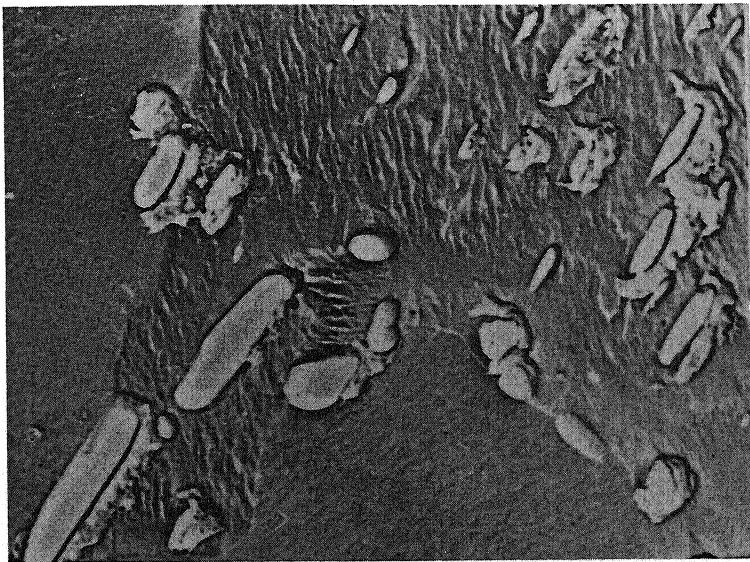


FIG. 195. Cartridge Steel. Taken by the electron microscope at 60,000 volts. Magnification 60,000 \times . (Courtesy, Philips Electronics, Inc.)

pearlite than is revealed in Plate XXXVII at 3000 diameters, or of martensite than can be understood from a study of Plate LI at 4500. Additional demonstration of this fact will be evident from a comparison of Plate LII showing a hypereutectoid steel at a magnification of only 2000 \times with a similar steel shown in Figure 195 which is at 60,000 \times . The more contrasty appearance at 60,000 \times is achieved by shadowing, which is almost necessary with a replica, but is not ordinarily done for study under a light microscope. Similar effects can be accomplished when desired, by the use of conical illumination. Moreover, with the light microscope additional information can be

gained by special staining methods and etchants, heat tinting, polarized light, and polished-surface identification of certain constituents. Hence the light microscope will always hold an important place in metallography.

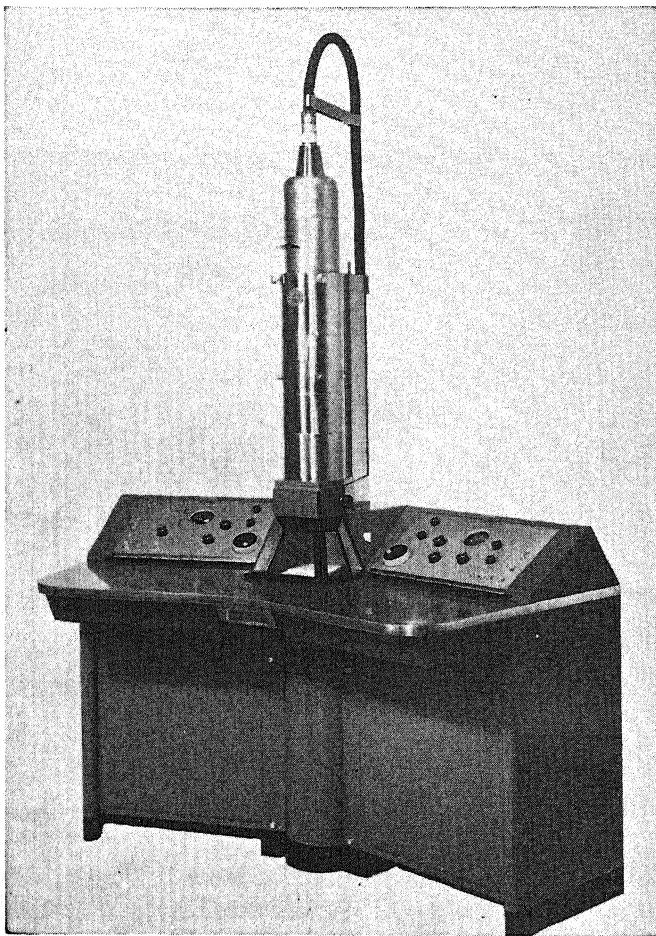


FIG. 196. The R.C.A. Electron Microscope; Model EMU-3

One of the inherent drawbacks in the electron microscope lies in the fact that the cathode rays can be propagated only in a vacuum. The entire system from the cathode-ray source to the fluorescent

screen or photographic film must be in a vacuum. This includes the object as well. The vacuum must be broken every time an object is inserted or removed, and the admitted air pumped out again. In some instruments the entire system is opened; others have means whereby only the specimen chamber is involved. This latter arrangement allows the time required for exhausting the air to be reduced from several minutes to a few seconds.

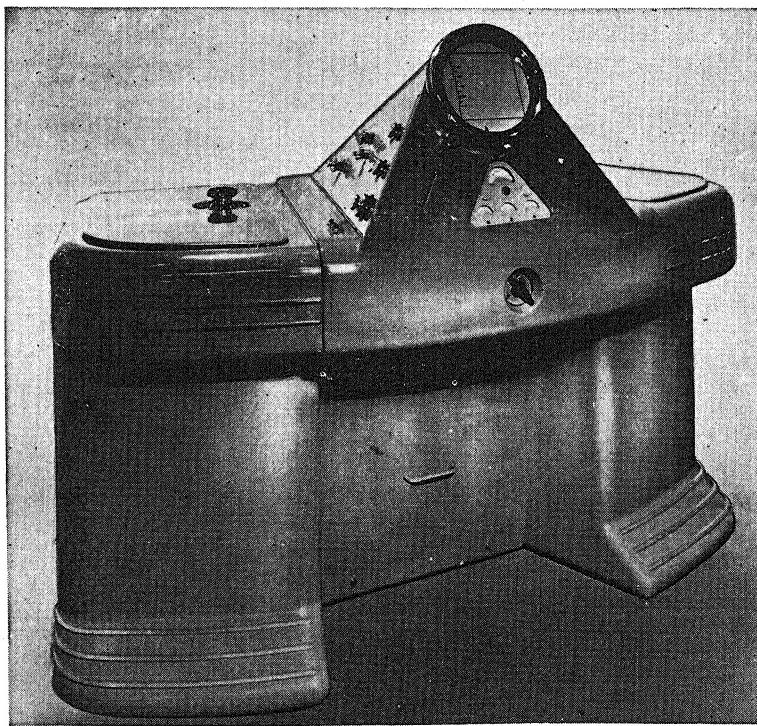


FIG. 197. The Philips Electron Microscope, Model EM-100

Objects (always very minute) are mounted on a thin film of collodion or other material fairly transparent to cathode rays; then this film is supported on a grid on the specimen holder for introduction into the microscope. The supporting film is made by dropping a minute quantity of a solution of the film material on distilled water where it immediately spreads to an almost monomolecular film. This is then lifted from the surface of the water, punched to fit the grid and mounted on the latter.

Changes in magnification are achieved by either varying the lens distances, changing the lens system, or varying the exciting current to the magnetic lenses. Radical changes in mechanical design have been made since the original instrument of R.C.A.* manufacture was placed on the market and several firms are now producing electron microscopes. The current design of the R.C.A. high-power model (EMU-3) is shown in Figure 196. This model can be operated at

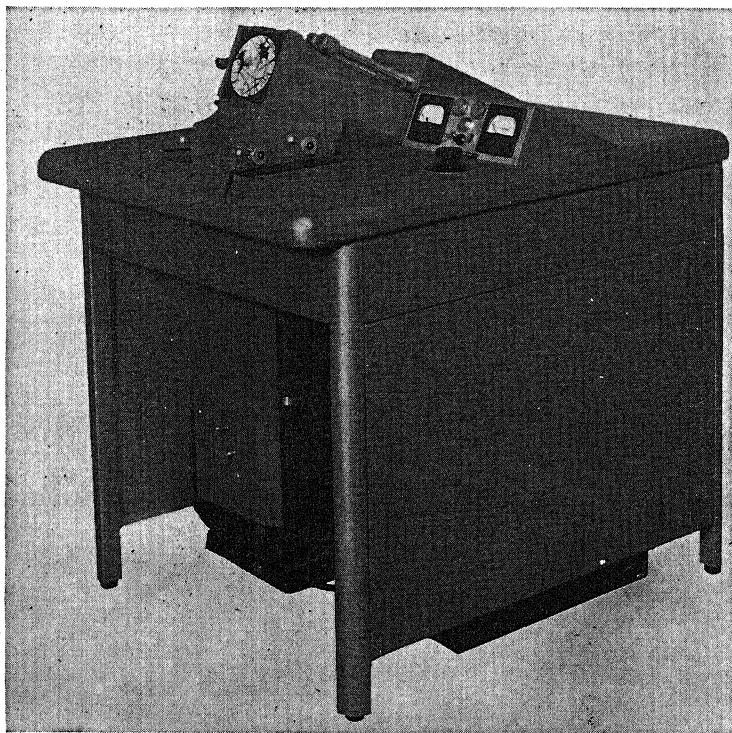


FIG. 198. The Farrand Electrostatic Electron Microscope, Model EST-1

either 100,000 or 50,000 volts. A less expensive model (EMU-1) of similar appearance is available in which the operating voltage is limited to 50,000 volts. The former has a resolution of 20 a.u. and direct visual magnification up to 50,000 \times ; the latter has limits of 30 a.u. and 30,000 \times visual magnification.

* The Radio Corporation of America, Engineering Products Division, Camden, N.J.

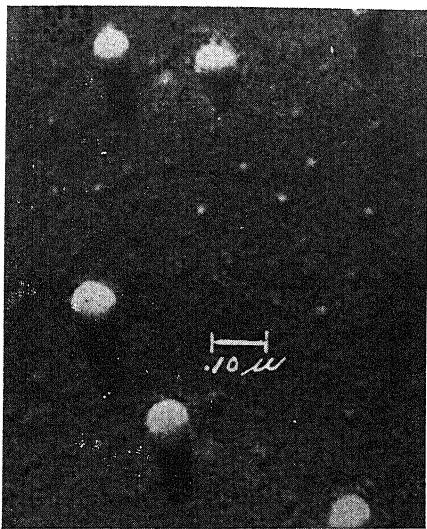


FIG. 199. Influenza Virus, Shadowed under the R.C.A. Electron Microscope. Magnification 58,925 \times . (Courtesy, Drs. Williams and Wyckoff, University of Michigan.)

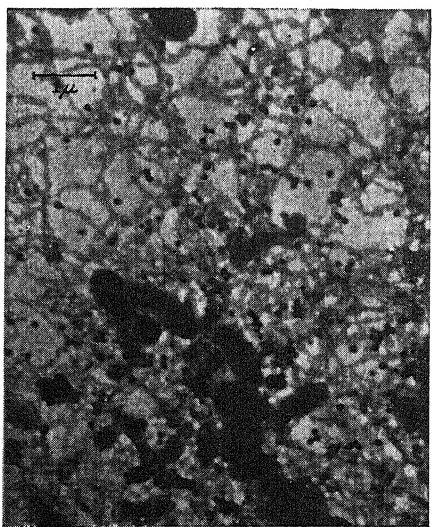


FIG. 200. A Tumor Cell from a Mammary-Gland Carcinoma, Magnified 7,875 \times under the R.C.A. Electron Microscope. (Courtesy, Drs. Porter and Thompson, Rockefeller Institute.)

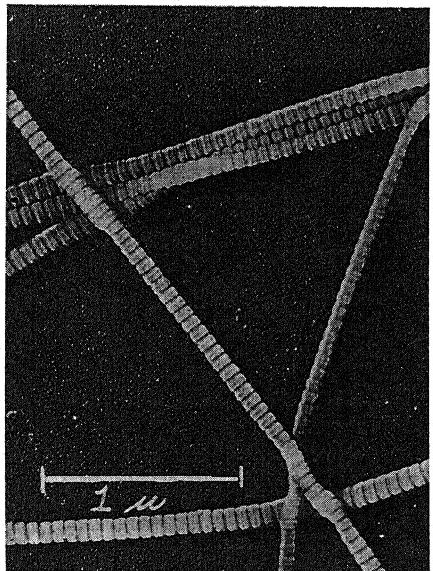


FIG. 201. Human Collagen, Shadowed with Chromium, under the R.C.A. Electron Microscope. Magnification 25,715 \times . (Courtesy, Drs. Gross and Schmitt, Massachusetts Institute of Technology.)

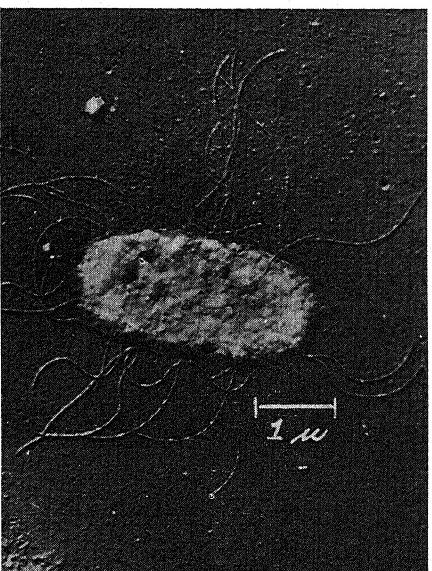


FIG. 202. A *Proteus* Bacillus under the R.C.A. Electron Microscope at a Magnification of 10,200 \times , Showing Its Many Flagella. (Courtesy, Dr. R. Wyckoff, National Institutes of Health.)

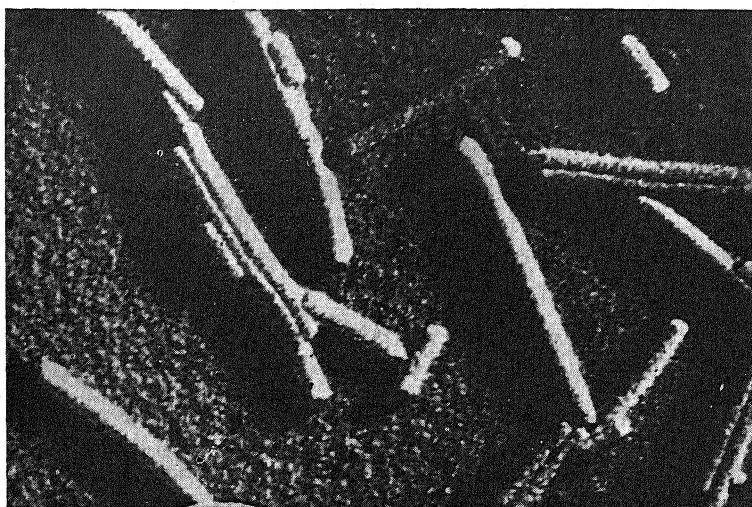


FIG. 203. Tobacco Mosaic Virus under the Philips Electronic Microscope at 100,000 Volts and Magnified 200,000 \times . (Courtesy, Philips Electronics, Inc.)

Philips Electronics, Inc.* Model EM-100 is shown in Figure 197. It also is a high voltage outfit, operating up to 100,000 volts. It differs from the R.C.A. design in that the microscope tube is set at an inclined angle and viewed directly in front, in a manner similar to a TV screen.

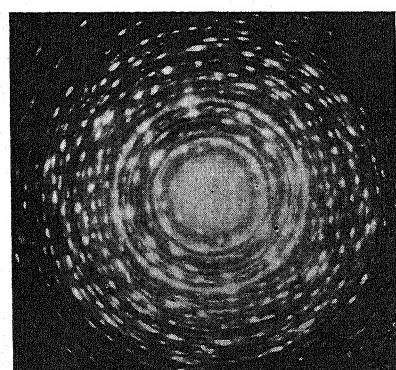
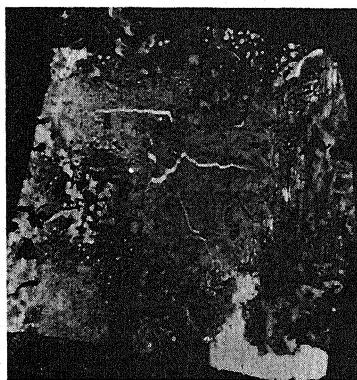


FIG. 204. *Left:* An area of oxide scale in stainless steel, three-fourths of a micron square, selected to undergo diffraction study. *Right:* The diffraction pattern. Philips Electron Microscope, at 100,000 volts. (Courtesy, Philips Electronics, Inc.)

* Address: 750 South Fulton Avenue, Mount Vernon, N.Y.

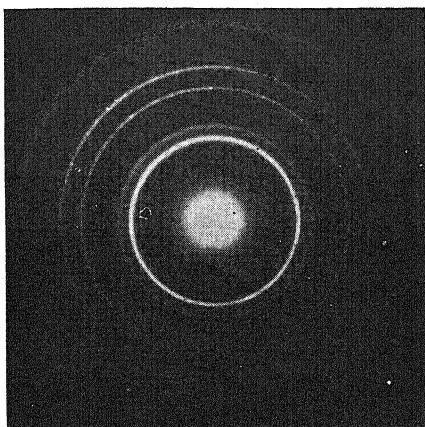


FIG. 205. The Diffraction Pattern of a Colloidal Gold Film on Collodion Seen in an Electron-Microscope Photomicrograph. (Courtesy, Farrand Optical Company.)

The electron microscope (Model EST-1) manufactured by the Farrand Optical Company* is shown in Figure 198. It follows the inclined-tube design with a direct-vision screen. While it operates on a lower maximum voltage, it yields a resolution of 30 a.u. and a direct magnification on the viewing screen of 20,000 \times .

All three of these microscopes are arranged to swing over to show diffraction patterns. Stereoscopic micrographs can also be taken on all of them. To accomplish this it is necessary to take two separate

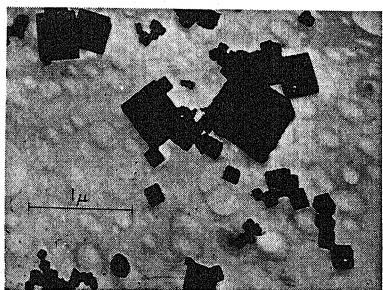


FIG. 206. Magnesium Smoke under the Farrand, Electrostatic Electron Microscope. Magnification 30,000 \times

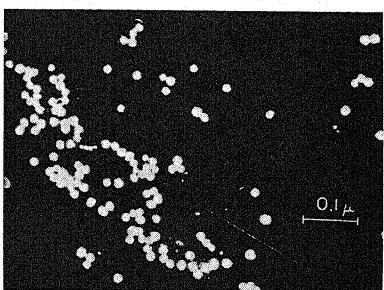


FIG. 207. Colloidal Gold under the Farrand Electrostatic Microscope. Magnification 150,000 \times

* Address: Bronx Boulevard and East 238th Street, New York 70, N.Y.

negatives with the specimen moved into angular positions comparable to the parallax of vision present in the human eyes.

Figures 199 to 207 show micrographs of various objects at a wide range of magnifications, made on the various models, as well as the nature of diffraction patterns secured with electron microscopes. The magnifications listed belong to the original photographs. These have been considerably reduced for reproduction in these pages.

Microphotography

The term *microphotograph* is frequently employed, even by some microscopists, as the equivalent of *photomicrograph*. This is incorrect; the word means "a minute photograph."

Photographs reduced to microscopic size, capable of being seen in detail only under a microscope, are not novelties, for they have long been in practical use. They were of great value in the siege of Paris, during the Franco-Prussian War of 1871, when, by the employment of the Pigeon Post film, messages were passed back and forth between the besieged city and the outside world. The messages were first printed in newspaper form and then reduced to minute photographic copies on celluloid film. These films were rolled into small tubes and attached to the leg of a homing pigeon. When received they were read by means of a microscope. Although these hastily executed miniature publications cannot rank as works of art, it is remarkable, considering that photographic technique some ninety years ago was not so nearly perfected as it is today, how legible the copies were. Figure 208 shows a micrograph made from an authentic piece of this Pigeon Post film, at a magnification of 50 diameters.

Many improvements in microphotographic technique have been introduced since the days of the Franco-Prussian War and some wonderful commercial work has been done. Until recent years, however, most of it has been confined to the production of microscopical slides of microphotographs. These illustrate to the amateur microscopist the art of microphotography; examples can be found in the slide collections of the majority of the older generation. A photomicrographic copy of one of these microphotographs is shown in Figure 209. The original measures .77 mm. by 1.1 mm. (less than $1/16''$ high) and it is magnified 85 diameters in the micrograph.

They have also been exploited as novelties, in the form of a minute hollow-tube holder in which is placed a micrograph mounted on the end of a glass rod with a strongly magnifying lens at the opposite end. The enlarged image is seen by looking into the eye end of the tube,



FIG. 208. Photomicrograph of Pigeon Post Film, Paris 1871, 50x

with the tube pointed toward a bright light. From the strictly scientific standpoint, microphotography has been of value in the production of minute instrument scales, stage micrometers, eyepiece disks of various types, etc.

In recent years there has been a gradual application of the funda-

mental idea of microphotography to library and commercial problems. Maintenance of a complete file of records, newspapers, books, and other documents has grown to such tremendous proportions that storage room cannot be provided for an unlimited continuance of the practice. Reduction of such printed material to minute dimensions by means of microphotography has been found to be a practical solu-

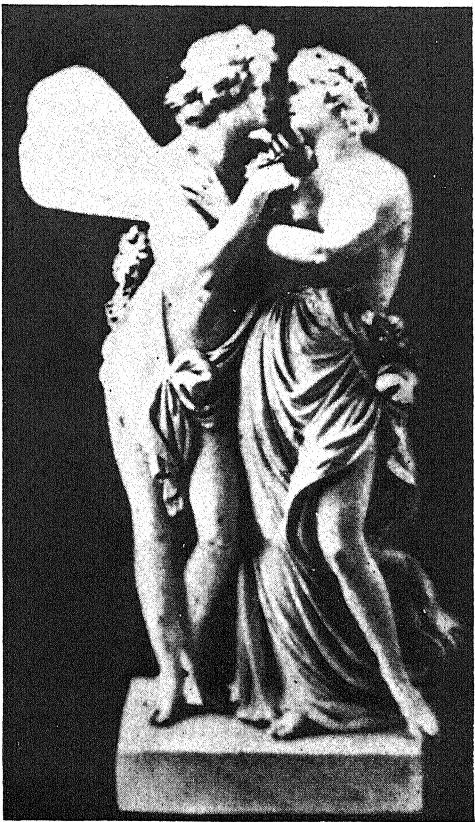


FIG. 209. Copy of a Microphotograph "Zephyr and Flora," 85x

tion. By this means a ten-thousand-volume library can be reduced to the size of a few ordinary books, and a complete newspaper is as easily stored as a postage stamp.

The microscope can lay claim to microphotography as its legitimate offspring, because some form of microscope is essential for the examination of a microphotograph after it has been produced.

For the examination of microphotographic copies of books, papers, and the like, numerous viewing devices have been and are still being developed. The more elaborate of these restore the copy to the full size of the original and are fully automatic in operation, to the extent that an entire book may be read through, just as easily as though it were a full-size library edition. An entire page is projected on a suitable viewing screen so that it is all visible at once.

The introduction of V mail during World War II, to expedite the handling of the great volume of letters to men overseas, was an added spur to the development of commercial equipment for producing reduced-size photographs.

Among others, the Eastman Kodak Company pioneered in design-

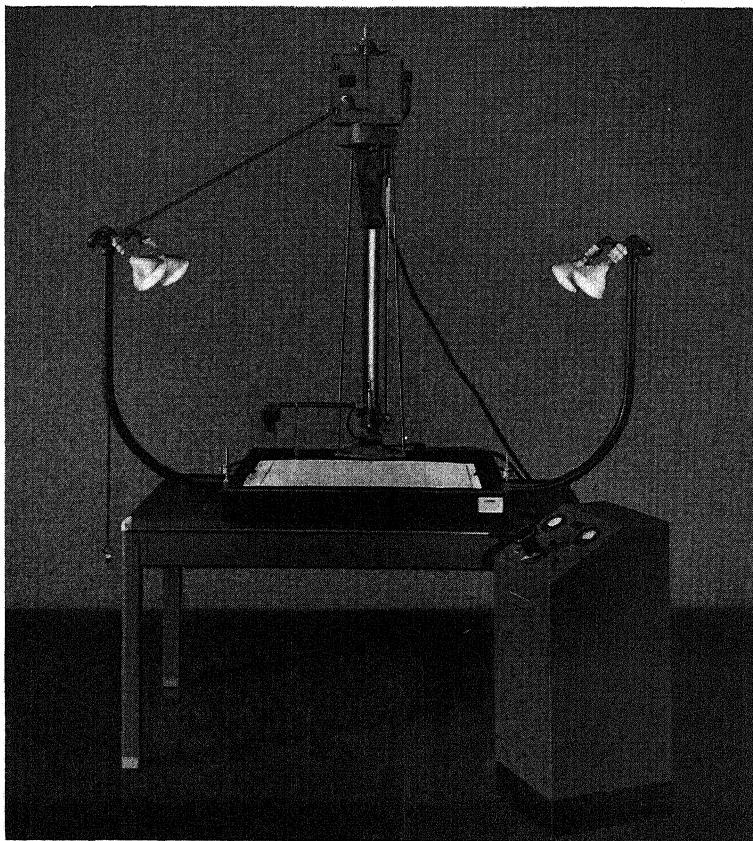


FIG. 210. Kodagraph Micro-File Machine, Model D

ing such equipment and placing it on the market. The term "microfilming" has been coined to cover the technique involved. Microfilming is now being done by practically all types of business and industry where abundance of correspondence, records, drawings, blueprints, sketches, etc., create problems of space for storage. As a result, the U.S. Bureau of Standards and the American Standards Association have issued standard specifications to unify the practices, techniques, film reduction sizes, and the like. The standard size employed for microfilming is 35-mm. film; for those who desire to employ 16-mm. film, adapters are provided for microfilming outfits to take this film. For some types of work, 70-mm. film is also standardized.

The Eastman Kodak Company supply three models of Kodagraph microfilming machines. These differ in the size of the surface to be reduced ($18'' \times 24''$, $24'' \times 36''$, and $37\frac{1}{2}'' \times 52\frac{1}{2}''$) and the amount of reduction provided for, which extends up to thirty times. Figure 210 shows their $24'' \times 36''$ Model D Microfile Machine. Kodagraph film readers are also supplied by Eastman in several models. Figure 211 shows the Model C Kodagraph Reader, which has an $18'' \times 18''$ viewing screen.

There are several other manufacturers supplying microfilming equipment, all, of course, based on the same general principles. Eastman does not manufacture equipment for 70-mm. film.*

Those interested in the commercial use of microfilming should

* Equipment for 70-mm. film can be obtained from Microtronics Corporation, 253 West 64th Street, New York 23, N.Y., and Photographic Products, Inc., 6916 Romaine Street, Hollywood 38, Calif.

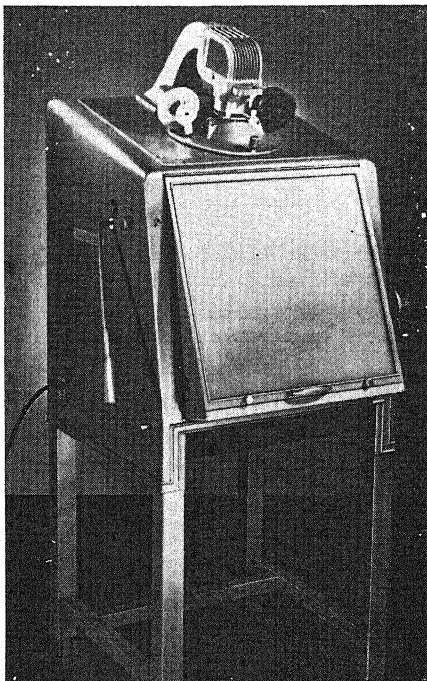


FIG. 211. Kodagraph Film Reader, Model C

secure a copy of the Eastman Kodak Company's booklet, *Microfilming with Kodagraph Micro-File Equipment and Materials*, which can be obtained for a nominal charge from camera supply houses. This contains full information on machines, processing techniques, special films for microfilming, and much other essential and valuable information.* The amateur microscopist, however, interested solely in the process as a means of making microphotographs on standard 1" x 3" microscope slides, needs very little apparatus beyond his standard microscope equipment. All this can be — or rather, must be — home-made, as there is no standard outfit on the market for this purpose. Thus very little expense is involved.

The making of microphotographs is an interesting side line for the photographically minded amateur microscopist. Probably much more would have been done before now, but unfortunately very little has been published about methods and there appears to be a common fear that the process is too complicated to be undertaken by the unskilled. Actually it is extremely simple and can be mastered by anyone sufficiently interested to take the steps involved in setting up for the work. A brief description of methods is included here so that all phases of photographic work in which the microscope plays a part can be said to be covered.

In principle, the making of microphotographs does not differ from the making of lantern slides from original negatives of larger size. It is essentially the degree of reduction that is involved, but because the reduction is so extreme in amount, and the finished transparency so minute, three new factors are involved in the production of first-class pictures. These are: (1) the quality of the lens which must be employed in the reduction of the negative; (2) the need for accurate focussing, and (3) the use of a transparency plate which has a substantially grainless emulsion when properly developed.

(1) *The Lens*

It is obvious that the limit of 250 microns on the size of the circle of confusion, ordinarily considered satisfactory for photographic purposes, is absolutely impossible in microphotographic work. When the reproductions are unusually small, a magnification of 100 diameters may be required to show them properly. If the pictures are to be clear and sharply defined at this magnification, the 250 micron limit

* A recent book on the subject is *Microrecording* by Chester M. Lewis and William H. Offenhauser, Interscience Publishing Co., 1956, \$8.00.

cannot be greatly exceeded in the *magnified image*. This means that the circle of confusion in the microphotograph itself cannot exceed more than a few microns in diameter.

Fortunately the microscopist usually has, in his standard equipment, lenses which should meet this requirement. These are his low-power objectives, and microphotographic lenses. The latter are, for reasons which will be evident later, to be preferred where they are available. Naturally the smallest microphotographs can be produced with the shortest-focus lens, other conditions being equal. As explained in Chapter 1, unsymmetrical lenses must be employed in the way in which they are designed to operate. Hence it is important, whatever lens is used, to have the conjugate foci of the lens which has been designed as the shorter pointed toward the *image*, when the latter is a reduced image, as is the case when making a microphotograph. With low-power microscope lenses, and microphotographic lenses (Micro-Tessars, etc.), this means that the front of the lens, which is toward the object in taking a photomicrograph, must be faced toward the *plate* in making a microphotograph.

Should one contemplate buying a lens especially for microphotographic work, the best is a high-quality motion-picture lens such as those used on 8-mm. cameras. It need not necessarily be a lens with a high aperture; preference should be given to that for which the smallest circle of confusion is claimed by the manufacturer. A lens of this type will be faced toward the negative which is to be reproduced, and the plate will occupy the position of the motion-picture film.

Ordinary microscope objectives up to 16 mm. (10 \times) can be used, but generally the image will not be ideal. The reason is that such lenses are corrected for use with a cover glass and for a definite tube length. To obtain the best result the negative should be located at the distance of the eyepiece diaphragm from the back of the lens, and the picture should be taken with a cover glass in front of the sensitized film, which would occupy the position of the object, in relation to the objective. Omission of the cover glass adds to the tube length (i.e., the position where the negative should be situated), but the latter would still be too close for practical work, because theoretically it would require a negative only ten to twelve times the size of the micrograph to be made from it. With a lens not exceeding 10 \times , however, considerable leeway will exist, even when ideal conditions are not present, so that fairly good pictures can often be obtained by observing the conditions outlined later on. Objectives corrected

for a tube length of infinity will be found to work much better. Many such are now provided for use on special equipments. With ordinary objectives a partial correction can be effected by the use of a negative spectacle lens of one or two diopters, located just in front of the objective (i.e., actually at the back of the lens, as it would be in the microscope which becomes the front in taking micrographs). A negative lens extends the tube length, giving an approximation of the desired longer conjugate focus. Blank (circular cut) spectacle lenses usually can be secured from an optician or eye specialist. Such a lens must of course be a simple negative lens, without any correction for astigmatism.

(2) *The Need for Accurate Focussing*

The fact that the microphotograph is, in effect, a microscopic object which must be examined at considerable magnification, is sufficient explanation why a perfect focus must be secured before the exposure is made. A safe rule to follow is that the image of the negative should be observed while being focussed on the plane of the film, at the magnification which will be used later on to show the finished micrograph. For instance, if the size of the image is such that it just nicely fills the field of the microscope at 100 diameters, it should be examined and focussed at this magnification. Logically this implies that the entire process be carried out *under a microscope*. This is the crux of the entire process. The practical working out of the method by which it is accomplished will be outlined further on.

(3) *Suitable Plates for Microphotographs*

The third imperative condition to be met in the production of microphotographs is the practical elimination of grain in the plate. Ordinary plates are not suitable, for the sensitive silver emulsion, instead of being homogeneous (or rather, so finely emulsified as to appear homogeneous, at fairly high magnification), is very grainy, the silver being present in the finished negative in minute blackened particles which produce the image. Hence the image, although satisfactory at normal size, or slightly enlarged, is hardly recognizable when highly magnified.

A general law relating to graininess in ordinary photography is that fast (or very sensitive) emulsions are inherently coarser than slow

ones. This implies that the very slow process, or lantern-slide positive, plates can be expected to represent the finest possible grain to be found in commercial plates, which is true. Much has recently been done, largely because of the popularizing of miniature cameras and the introduction of reversible 16-mm. motion picture film, in the reduction of grain size in fast and supersensitive emulsions. Reduction of grain size has not been carried out to the same extent on very slow emulsions, largely because there is no need for finer grain in this range in ordinary photographic work.

The work on emulsion grain size has been supplemented by the introduction of fine-grain developers. Where necessary, the use of fine-grain developers on slow emulsions gives as grainless a transparency or positive reproduction as can be desired for all usual purposes.

Such fine grain is, however, not suited for microphotographs. With ordinary process plate emulsions, even if the graininess could be completely eliminated through proper development, other conditions are present which make them unsuited for microphotographic work. These are the thickness of the emulsion, and its turbidity. These two factors, operating together, result in a spreading of the light, so that, microscopically speaking, a diffused image results.

Fortunately, for the microscopist desirous of experimenting with microphotographic work, there is available a commercial plate which, for all practical purposes, is suitable, provided magnifications not in excess of 100 diameters are used for viewing the microphotographs. This is the Alpha Lantern Slide plate, manufactured by Ilford, Ltd., London. The emulsion on these plates is extremely slow, very thin, and almost transparent. They should be developed in the exact developer recommended by the manufacturer. These plates are stocked by many of the larger photographic supply houses in this country.

The only commercial plate of American manufacture, of which I am aware, which comes into the grainless class of the Alpha plate is the Eastman Spectrographic plate, Type V. These plates must be ordered specially from the factory. They are, like the Alpha plates, extremely slow, and as they are intended primarily for spectrographic work, are extremely contrasty. By overexposure and soft development they will suffice for microphotographs where contrast is not objectionable, and are especially fine for ruled scales.

The ideal emulsion for this type of work is one of the old-style

albumen emulsion, or next best, the collodio-bromide type of plate, for these are substantially grainless. As these are not made commercially, the entire process of making the emulsions, coating the plates, and preparing them for use must be performed as a part of the work. This handicap is likely to dampen the ardor of the most enthusiastic microscopist. If, after experimenting with Alpha plates, one enjoys the hobby sufficiently to go the limit, he can then take up the coating of his own plates. Formulae for such emulsions can be found in many works on photography.

The Method of Making Microphotographs

Only two pieces of equipment are essential to the process. These are the microscope and the illuminating device for the negative. The latter can be designed along any one of several lines, but must be, to some extent at least, homemade. From the standpoint of ultimate effect, it can be described as a light source, in front of which is placed the negative, the whole suitably enclosed so that no light, other than that passing through the negative, is visible.

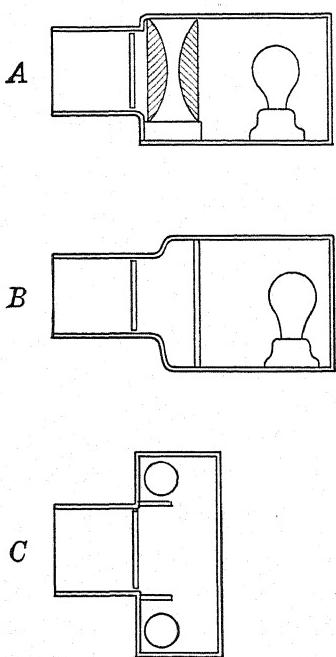


FIG. 212. Designs of Lamp House and Negative Carrier for Taking Microphotographs

light efficiency, but if the condensers must be purchased especially for the purpose, it is the most expensive of the three designs.

Figure 212 shows three possible arrangements, all equally satisfactory. From the operating point of view, they differ only in the relative intensity of the light available, which affects the time of exposure. The exact time with any of these systems can be varied over a considerable range by merely changing the power of lamp employed, and hence that design can be chosen which will be the easiest for one to construct with the facilities at his command.

At A is shown the conventional condenser method, as used for stereopticon projection. This provides the highest

The design shown in *B* is next in efficiency as to light intensity, and is satisfactory when the negative is not large. A large negative may require more than one lamp to provide uniform illumination, or the single lamp must be placed at a greater distance from the opal glass. Unless a great distance can exist between the lens and negative there is not much chance of a large negative's being satisfactorily copied. The efficiency of this design is increased by painting the entire inside of the housing with flat white paint.

The indirect illumination used in the design shown in *C* provides an ideally uniform lighting of the negative, but the efficiency is low and longer exposures are required.

Whichever design is used should provide for easy insertion and removal of the negative and should have the light shield in front of the negative extended sufficiently far to reduce the stray light entering the room. Either wood or sheet metal can be employed to make the housing.

Where possible, a uniform negative size should be established, since this simplifies the design of the apparatus and the entire setup need

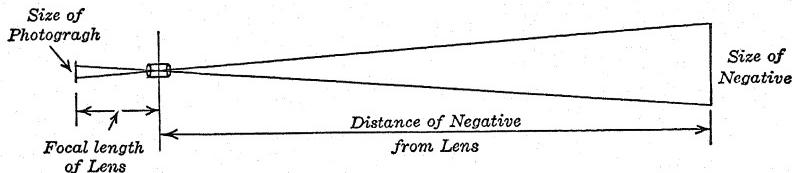


FIG. 213. Relation of the Objective Focal Length and Negative Distance, to the Size of Negative and the Resultant Microphotograph Size

never be changed, except for a possible variation in the distance of the negative from the lens, when larger or smaller pictures are desired.

When a miniature camera of the 35-mm. type is available, this size of negative will be found ideal as a standard size for all work. The actual holder for the negative can be made to accommodate a larger size than this — possibly up to the size of a No. 120 (Brownie Kodak) film. The wisdom of exceeding this latter size, however, is questionable, for usually it will be found that the practical distance which can be provided between the negative and lens limits the negative size materially. Before deciding upon the size to be used, it is well to take stock of all factors having a bearing on it.

Figure 149 illustrates diagrammatically the relationships involved. Working out a practical example according to this diagram, if we

assume a lens of one-inch focal length to be used, and a picture one millimeter square to be desired, a negative $1\frac{1}{2}''$ square would need to be placed three feet from the lens, to secure the proper result.

Whatever size of negative is used, it must be so framed in the holder that no extraneous light can pass it. The framing actually forms the limiting borders of the microphotograph.

Although the microscope is an essential part of the equipment, the pictures are not made *through* the microscope, which is employed primarily for viewing and focussing the image. The lens used for making the pictures is to be mounted in the substage condenser ring, in place of the regular condenser. Usually some form of adapter must be made specially for this purpose, but it need not be expensive. Where Zeiss microscopes are available, the centering objective holder for the substages, which is standard equipment with this company, can be used for all lenses equipped with the Royal Society screw thread. Lenses with other threads must be fitted with adapters to fit the objective holder.

Those who have mastered the principle of critical illumination will quickly grasp the idea of the entire process. The microscope must be placed in its horizontal position and the illumination box with the negative in position, emulsion side out, must be located in the optic axis of the microscope, the proper distance away, to yield the desired

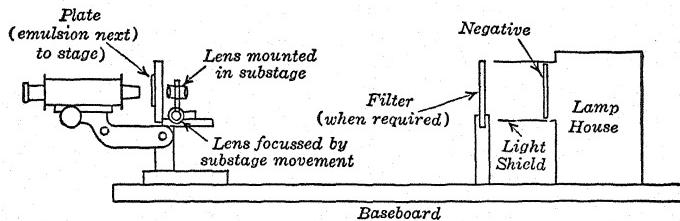


FIG. 214. Diagram of Complete Setup for Taking Microphotographs

image. Though a special base or support for the two pieces of apparatus is not essential, it simplifies matters appreciably, if much work along this line is contemplated, to provide a heavy board with guide strips and clamps, for this purpose. The setup is shown diagrammatically in Figure 214.

The microscope stage is the support for the plate on which the picture is to be taken. The emulsion side is placed directly against the surface of the stage. This means that the focussing of the

microscope on the plane of the image must be done through the thickness of the glass plate. As any variation in this thickness affects the focus, it is important that the focussing be done in every instance on the exact plate on which the microphotograph is to be made, but usually one focussing operation will suffice for all exposures made on the same plate. The particular method of focussing to be adopted will depend upon whether an entire plate 3" x 1" is used for each picture, or several are taken on a strip which are subsequently cut up to be mounted on ordinary glass slides. The latter is by far the less expensive, but does not make as neat a finished slide, and involves more work in finishing the slide.

If the plates are purchased in the standard $3\frac{1}{4}$ " x 4" lantern-slide size, they must be cut to the proper size by means of a glass cutter (preferably a diamond) as the first operation. This must be done in the darkroom, but a fairly bright orange light can be employed. The Eastman #0 safelight is suitable. If 3" x 1" slips are to be used, the first cut should be a $\frac{1}{4}$ " strip off the $3\frac{1}{4}$ " dimension, to reduce the plate to 3" x 4". This $\frac{1}{4}$ " strip should be saved for focussing purposes. It can also be used for making test exposures. The 3" x 4" glass is then cut into four 3" x 1" strips; these are packed away in a box until they are required, to prevent fogging. With this method each original lantern slide will produce four finished slides.

If the alternate method of making separate small pictures is to be used, the lantern-slide plate can be cut into six strips, along the four-inch length. These strips should not be cut into the smaller squares until after the pictures are finished. Each strip will make at least four, which will give twenty-four possible pictures to a plate.

Having provided plates cut to the desired size and aligned the apparatus, we are now ready to proceed with the actual work of making the exposure.

The room in which the work is being done must be capable of being darkened to exclude all light that would fog the plate, as the latter is not mounted in a holder of any sort, but placed directly on the stage of the microscope, film side down. A ruby or orange bulb in one of the light fixtures will provide ample but safe illumination, as the plates are very insensitive to any but the blue rays. (This does not apply, of course, to the Eastman spectrograph plates, Type V.)

In placing the plate in position on the stage care should be taken not to slide it on the emulsion, as scratching of the film may result. Each time it is moved, it should first be raised from the stage. With

the 3" x 1" slide plates, focussing can be done on either end, or anywhere except within a $\frac{3}{4}$ " circle in the center, as the emulsion will later be removed from the slide in all but the central area.

With a plate in position on the stage, the first operation is the focussing of the microscope on the emulsion. A 10x objective is preferable for this purpose. If any difficulty is experienced in finding the exact focus, a minute scratch with a needle will provide an object. When the $\frac{1}{4}$ " strip is saved it should be used for this purpose. After the microscope has been focussed on the film the focussing adjustments *must not be touched again* while slides from the same plate are being exposed. In doing this focussing it is desirable that the lens in the substage condenser, with which the picture is to be taken, be thrown entirely out of focus so that there is no temptation to focus on the image of the negative instead of on the emulsion surface. In other words, we must bring the image of the negative to the proper focus on the emulsion (which means bring it to the point of sharpest definition in the microscope) *after* the latter is properly focussed. This is accomplished with the focussing means provided for the substage condenser. Instruments provided only with slip-ring condensers will naturally be more difficult to adjust to proper focus.

In the diagram (Figure 214) a filter holder is indicated, located directly in front of the negative. If the correction of the photographic lens is not so perfect as it should be, the blue rays, which are the only ones affecting the plate, may not possess the same focus as the optical center of white light. When this is the case, the introduction of a blue filter (of optical quality) will aid in obtaining the proper focus for the blue rays. The filter should then be left in for taking the picture.*

Having obtained the proper focus of the negative on the plate, the next step is the making of test exposures, which should be made in geometric progression, 1, 2, 4, 8, 16, etc. It may even be necessary to make a second set of test exposures, and finally to change the lamp so that the proper time may fall somewhere between ten and sixty seconds. For making the test exposures it is permissible to slide the plate along, for on test plates scratches are immaterial. The test

* This applies to the Alpha plate and special albumen or collodion emulsions. If the Eastman Spectrographic plate, Type V, is used, with the usual panchromatic sensitizing, it will register all the visible light. Nevertheless an improvement in the image may result, even in this case, through the use of a filter giving a narrow band. It need not necessarily be in the blue, however; a green band may be still more effective.

exposures can be spaced about $\frac{1}{4}$ " apart, in order that a considerable number may be possible on a single strip. Examination of the wet strip, after developing and fixing, will show the proper time.

When 3" x 1" slips are used, the picture should be located in the center. Small squares should be so positioned on the strip that a single cut between each picture will result in a neatly centered object.

After the plates are dry, the pictures should be covered with a cover glass and Canada balsam (in xylol), and set aside until thoroughly hard. The edges of the slide can then be ground smooth on a coarse India or carborundum hone, using thin oil or turpentine. The final step is to scrape all the emulsion from the uncovered portion of the slide, and then clean and label.

With small squares the additional work involved lies in cutting the individual pictures apart, grinding the four edges of each, and then cementing them to standard slips with balsam.

The entire process can be a fascinating hobby and many pictures can be produced as a result of an evening's work.

More seriously, the making of special scales for various scientific measuring instruments is frequently called for. Such scales or ruled designs must be accurately drawn in ink on white cardboard and then photographed on the proper-size negative.

No attempt need be made to keep the scales to a definite size in the negative, as final adjustment in this case is to be made by altering the distance of the negative from the lens until the image measures the proper size. To effect the measurement of the scale on the microphotograph, an eyepiece micrometer must be employed in the microscope. This micrometer must be accurately calibrated beforehand, by means of a stage micrometer.

Finally, mention should be made of what might appear to be the simplest and most logical method for the amateur microscopist of making microphotographs. This is to take them on 8-, 16-, or 35-mm. film by means of a standard miniature camera. This yields passable results, not at all ideal. It presents some complications which must be recognized.

In the first place, while microfilming is confined to about 30 \times reduction, the microscopist usually wants a picture which can be viewed at around 100 diameters and yet be extremely sharp. When one thinks of the enlargement of an 8-mm. film on a screen to possibly several feet, and apparently fairly sharp when viewed, he does not consider that the magnification may be only around 50 \times and that it is

viewed from a distance of several feet instead of from 10 inches, as it would appear under a microscope at this magnification. While the graininess of modern films has been greatly reduced, it still is not able to stand an enlargement of 100 diameters.

Then again, the degree of reduction must be figured accurately in order to obtain the desired size of picture. For instance, if a 5" x 7" picture is to be copied, with the larger dimension reduced to $\frac{1}{16}$ inch, the ratio of the focal length of the camera lens (which must be known) to the distance of the camera from the picture should be roughly as 100:1. With a 1-inch lens this would mean the object picture should be $8\frac{1}{2}$ feet away.

A third factor is that the focussing of the camera must be exact for the distance, something difficult to determine from the engraved distance settings. Stopping down to $f:16$ will help, but the proper procedure is to test the focus with the back of the camera removed, substituting a fine ground glass for the film and focussing the lens wide open, with at least a 6x magnifying glass. Then stop down to $f:16$ and maximum sharpness should result.

Microphotographs must be made as positives; therefore if they are made on 8- or 16-mm. film, reversal is necessary when copying a picture unless a negative of the latter can first be made. If a negative is made, some reduction can be secured by this means if the original is large, thus providing a two-step reduction — a decided advantage where it can be accomplished.

Photographic Processes, Materials, and Equipment

The Chemistry of Development

The possibility of producing a permanent negative image through the use of various salts of silver is due to the fact that such salts are unstable under the action of light. They tend to break down into metallic silver, which, when dispersed in a solid emulsion, will exist as microscopic opaque particles.

When an intense light is allowed to act for a sufficient time on such an emulsion, the light alone is capable of producing reduction to metallic silver; but in some manner not yet fully understood, a faint light is also capable of initiating the process of reduction, the effect being proportional to the intensity of the light. The most plausible explanations appear to be either that atomic nuclei of metallic silver are originated by the light (their number and size depending on the light intensity), these nuclei functioning as attraction centers upon which additional silver is deposited by reducing agents, or that some weakening of the bond between anions and cations occurs which aids subsequent reduction by chemical means.

The particular silver salts of value in photography are those in which the metal is combined with one of the halogen group of elements, chlorine, bromine, and iodine. The incorporation of these silver compounds — silver chloride, silver bromide, and silver iodide — into the gelatin emulsion belongs to the plate- and film-maker's art; it is not necessary for the photographer to know the underlying chemistry, other than to know that plates and films can be produced which possess certain characteristics as to speed, softness, etc., in varying degrees. The one feature common to all, however, is their capacity to receive the latent image. This latent image, not visible in the undeveloped state, can be acted upon by certain chemicals until it blackens. The degree of blackening is proportional to the amount of light which was allowed to fall upon the emulsion. The process of producing the blackened image is called *developing*.

Great divergence in the sorts of objects which must be photographed so as to reveal their nature to the best advantage calls for considerable latitude in the development and subsequent printing processes. Or, to express it in photographic terms, some negatives must be developed for extreme contrast, others for maximum softness.

Although the desired result can be obtained in part by proper selection of the plate or film employed, in large measure the development can also be controlled to improve the quality of the negative. As development is a chemical process, an understanding of the chemistry of development helps to determine procedures.

The chemicals used in the production of the blackened image are employed in an aqueous solution, technically known as the "developer." This developing solution is not a single chemical, but a composite mixture of several, which in various proportions yield different results. A knowledge, therefore, of the part played by each constituent is useful to all who desire to become competent photographers.

The process of transforming the silver from the salt to the metallic state is known chemically as *reduction*. Any chemical which will bring about this change is called a *reducing agent*. Therefore, one constituent of every developing solution must be such a reducing agent. Many of these are available; most are organic compounds. But, for several reasons, none of these reducing agents is satisfactory for the development of an exposed plate, when used alone. In the first place, reduction will ordinarily take place only in an alkaline solution, although the reducing agents are either neutral or acid. Thus in addition to the active reducing agent (or "developer"), the solution must contain an alkaline substance as well. This is usually sodium carbonate, although other alkalis, such as the hydroxides (ammonia, for example), borax, etc., are also used. As the activity of the developing agent is somewhat proportional to the amount of alkali present, and as the more rapid the reaction, the greater the contrast, it will be apparent that, other things being equal, greater contrast in the negative can be secured by increasing the percentage of sodium carbonate, and greater softness by decreasing it. Sodium carbonate in the developer also acts to soften the emulsion so that the solution can penetrate it more readily.

Examination of published formulas will show that practically all developing solutions contain, in addition to the carbonate, sodium sulfite as well. This is added to prevent the developing agent from rapidly absorbing oxygen from the air. Oxygen will not only reduce

the solution's strength, but will discolor it to a dark brown, which can produce staining of the gelatin of the emulsion. A chemical which will prevent this is primarily a preserving agent. Sodium sulfite possesses a greater affinity for oxygen than the reducing agent, and hence keeps it from the latter. As a rule, those developers keep best which can take the largest percentage of sulfite without interference with the reducing action of the developing agent.

The other important constituent of developing solutions is potassium bromide. This is added only in small amounts and is termed a *restraining agent*. When it is not present, an emulsion will be more or less reduced, or darkened, even though it has not been exposed to any light. Such darkening is called *chemical fog*. It has been found that when potassium bromide is present in the solution, it restrains the general reduction and limits it to those portions which have actually been exposed to the light, so that the amount of darkening can be made to correspond to the degree of light acting on the plate.

Thus we see that (with few exceptions) a developing solution must consist of a combination of four chemicals: the reducing agent (or developer), an activator (sodium carbonate or other alkali), a preserving agent (sodium sulfite), and a restraining agent (potassium bromide).

The reducing agent can be any one of many which are commercially available for the purpose, or, often, a combination of two which possess different characteristics. By changing the proportions of the various constituents, it is possible to secure a wide range in the degree of contrast or softness. This process may apply not only to the development of the negative, but to the making of the finished paper print (or positive) as well, since the latter process is identical in principle with the development of the plate.

To enable photographers to secure the best results, the manufacturers recommend certain developing formulas for both plates (or films) and printing papers. It is a good plan to begin by following the recommendations of the manufacturers until one becomes thoroughly familiar with the principles involved.

The Chemistry of Fixation

Negatives retain in the emulsion undeveloped silver salts where only partial or no reduction took place; if these are not eliminated, reduction will continue to occur when the negative is exposed to light. Not only would such subsequent reduction be fatal to the negative, since it

would ultimately become completely black, but the presence of the unreduced silver makes the emulsion relatively opaque, whereas to produce a positive print, it must be transparent in unexposed areas.

For these reasons, before the developed negative can be exposed to the light it must be placed in a bath of hypo ("hyposulfite of soda," sodium thiosulfate). In this chemical all the undeveloped silver salts are dissolved out; the process is termed *fixation*. The reduced silver is not affected.

Although plain hypo will accomplish the fixation, fixing bath formulas usually call for additional chemicals. In so-called "acid hypo," these are generally sodium sulfite, acetic acid, and potassium alum.

The acetic acid is employed to neutralize the alkali introduced by the developer; the sulfite has a double function — to preserve against oxidation (as in the case of the developer) and thus prevent discoloration and also to prevent a chemical reaction between the acid and the hypo, which would cause free sulfite to separate out. The alum is used to harden the gelatin of the emulsion so it will not frill or fray during the subsequent washing it must receive.

The second stage of the photographic process — making the final positive print — is identical in principle with the production of the negative, so that fixation by means of hypo is required here also.

Before considering the actual technique of developing and printing, we should consider the equipment, materials, etc., required for the various stages of the work.

EQUIPMENT

(i) *The Darkroom*

All development must be carried out in the absence of any light to which plates or printing papers are sensitive; some form of darkroom is therefore essential. When only occasional work is done, darkrooms may be improvised in a closet, kitchen, or bathroom, especially if developing is done at night when no daylight need be excluded. Such a solution of the problem is a help to the amateur who otherwise might feel that the obstacles to taking up photomicrography were too numerous for him to overcome.

Where more serious work is contemplated, some form of permanent darkroom will prove valuable. It can often be constructed in an attic room or the cellar. Although it need not be large or elaborately furnished, the nearer it can conform, in general, to the ideal darkroom, as

required for semicommercial purposes or work on a larger scale, the more satisfactory it will be found to be. The requirements for an ideal darkroom are:

(1) The complete elimination of all extraneous light, combined with electrical outlet sockets for connection of lights, apparatus, etc., wherever required.

(2) Ample room for carrying on all the work which must be performed in it. At the same time, it should not be so large that needless steps must be taken during the various development processes.

(3) A large sink with running cold water (hot water is sometimes desirable, but is not essential).

(4) Ample bench room for the development stages. The bench should be reasonably close to the sink.

(5) Shelves for storage of developing equipment, chemicals, and photographic supplies.

(6) Suitable means of guaranteeing against inadvertent opening of the door or entrance of light during periods when plates or paper could be accidentally fogged and ruined.

(7) If the temperature of the room is likely to be raised unduly because of its location or confined condition, a ventilating system is almost a necessity.

As most darkrooms must be constructed with regard to space limitations, all will naturally vary somewhat in the way in which they are adjusted to these restrictions. It is, therefore, useless to illustrate the layout of a theoretically perfect arrangement. Furthermore, every worker will evolve his own ideas as to the best design. If the requirements listed are borne in mind in working out specific designs, the final results should prove satisfactory.

A few comments and suggestions, however, may help the worker to secure the best possible arrangement.

By far the best method for insuring safety against accidental opening of the door, either by the operator himself from the inside, or by someone else from the outside, is the use of a labyrinth entrance. Where there is ample room, the construction is probably no more expensive than that of a door; certainly it is less costly than a double-door arrangement.

A layout of such a labyrinth is shown in Figure 215. Where the light in the outer room is excessive, a movable dark curtain at the outer entrance may be required. With this type of entrance one need never fear accidental fogging of a box of plates or paper, or spoiling of a negative during development, by someone unwittingly entering

the darkroom. On the other hand, free passage in and out of the darkroom is always assured. This system also allows a better circulation of air, without resort to artificial circulation.

Opinions vary in regard to the finish of the darkroom interior. Three different colors are in common use — white (or light tint), black, and green. The argument for white is that if the safelight is really safe, whatever color is in use, white walls enable one to see better, and yet create no danger of fogging a plate or film. Green has

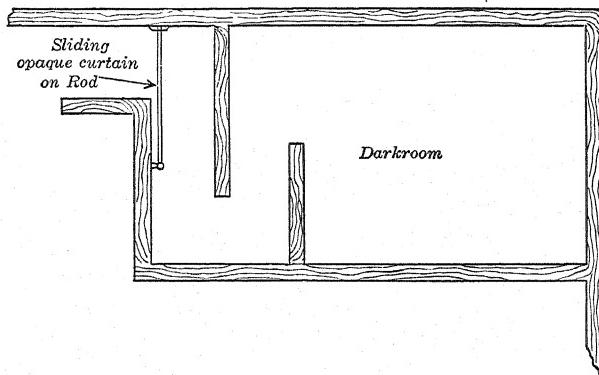


FIG. 215. Labyrinth Opening into Darkroom

the advantage that the eye is especially sensitive in this region, and hence can utilize light of an intensity to which most plates are insensitive, and the room appears much brighter than it actually is, photographically speaking. On the other hand, there are so many plates and films in current use which must be handled in absolute darkness, that the operator is compelled at times to know how to conduct all operations without seeing what he is doing. Hence the question naturally arises, "Why not carry on all operations under an absolute minimum of light and be on the safe side, in every kind of safelight, by painting the interior of the darkroom black?"

Where a labyrinth entrance is used, this at least must be finished in dead black; further, unless a curtain is always used in addition, a dead black finish is safer for the entire darkroom. As any light, however diffused it may be, which can enter by repeated reflection through the labyrinth, possesses rays which will fog any plate or film, light-colored walls in particular are undesirable. Where black is not used under these conditions, the green finish is preferable.

When space in the darkroom is a factor, it is sometimes important to know what apparatus can be used outside the darkroom and what

requires the pitch black of the darkroom. A very considerable part of the work of printing and enlarging can be carried out in the ordinary light of day as it enters a laboratory or basement area. Once the prints have been removed from the fixing bath, all further handling of them can be done in the light. The long washing in running water, if carried out in the ordinary darkroom, will seriously increase the dampness that so frequently adds to discomfort there. Squeezing prints, drying them, trimming them, and the like, are more handily done in the open where movement is unrestricted. There is no advantage in drying negatives in the darkroom. They are liable to dry very slowly in the still humidity that is usual there, and need to be hung where the air is at least in slight movement.

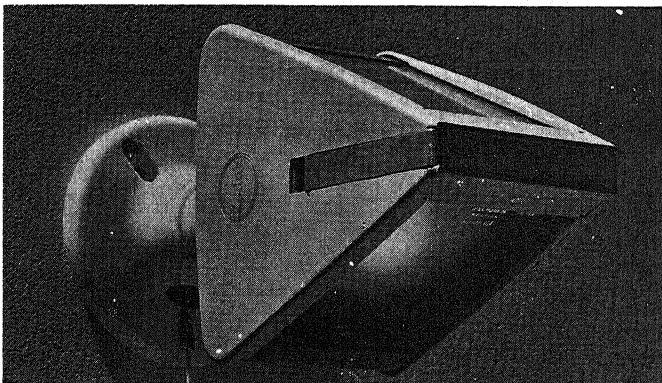


FIG. 216. Kodak Two-Way Safelamp

One of the easiest ways to relieve the strain on darkroom space is to arrange for darkening an ordinary room enough for certain kinds of work. For daylight hours the windows can be equipped with black shades, but not even this modified darkroom treatment may be needed if one works at night. In such a room the earlier stages of printing can be carried out as well as the later. In particular, enlarging, so often a problem with sizable apparatus, is far easier done outside the darkroom.

In general, as one stands while carrying on the various developing and printing operations, the height of the shelf should be suited to a standing instead of sitting position. This leaves considerable room underneath for shelves and storage space for trays and other equipment. Plates and materials liable to damage from solutions or wetting preferably should be stored in shelves above the bench, as accidents may allow seepage of solutions to storage places under the bench.

(2) Apparatus

The amount of apparatus required for development and printing processes is determined largely by the work to be done, the space avail-



FIG. 217. Enamelled Tray for Developing and Fixing

able both for using and storing it, and the size of the investment which can be devoted to it. On the one hand, the minimum, where work must be done in an improvised darkroom and everything packed away out of sight afterward, can be held to a safelight, three or four devel-

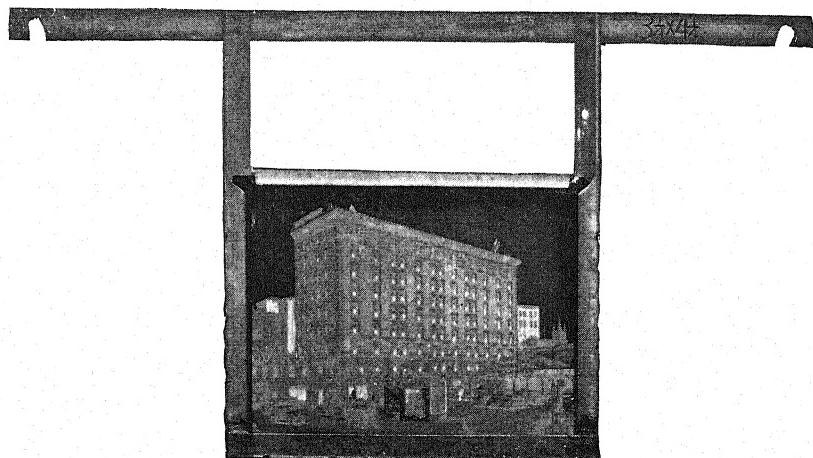


FIG. 218. Developing Hanger for Cut Film

oping trays (or a developing tank), and a printing frame. Standard developers and acid hypo are available in tubes and packages which require simply mixing with proper amounts of water for immediate use. Thus, equipment required for mixing one's own solutions and storing them in quantity is unnecessary.

At the other extreme is the completely equipped darkroom, with every device for expediting the work. Between these extremes lies a wide range where one may choose items of equipment as needed. Even though the start is made in a small way, growth can continue until facilities are ample to handle any problems one is likely to meet in photomicrography.

Some pieces of apparatus required for commercial developing and printing are not necessary for strictly photomicrographic work where only glossy papers are employed and retouching of negatives is not ordinarily permissible. Furthermore, it is hardly likely that mass production either in the development of negatives or the making of prints will ever be required of a photomicrographic darkroom. With these exceptions, the necessary equipment will not differ greatly from that employed for commercial photography.

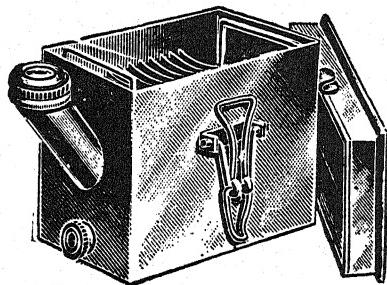
First on the list of indispensable equipment is a suitable safelight. Such lights are available in several types, according to the specific purposes for which they are required.

The name "safelight" must be flexibly construed, since a light which is perfectly safe for some work is not necessarily safe for others. Wherever limited light of a restricted color region must be employed, it is desirable that the maximum brightness consistent with absolute safety be used. No one light, therefore, will serve for all purposes.

FIG. 219. Tank for Plate or Cut Film

This requirement is met in well-designed safelights by the use of removable color screens which provide proper transmission characteristics. An inexpensive form is the Kodak 2-Way Safe Lamp pictured in Figure 216. The changeable screens (also designated safelights) are $3\frac{1}{4}'' \times 4\frac{3}{4}''$ in size. Number 0 is suitable for slow plates (e.g., process) and for bromide enlarging papers; #1 for non-color-corrected plates of any speed; #2 for orthochromatic emulsions not sensitive to red; and #3 for medium-speed panchromatic emulsions. High-speed panchromatic plates and films must be handled and developed in complete darkness.

In addition to the Kodak 2-Way Safelamp, Eastman have several other types to accommodate all possible conditions. These, together with a description of the various Kodak Safelight Filters are listed in their publication Kodak Pamphlet No. K-4, *How Safe Is Your*



Safelight? A copy of this pamphlet should be secured by all photomicrographers.

When one is mechanically inclined, it is a simple matter to construct a safelight box having advantages equal to any commercial safelights on the market. Any light-tight wooden or metal box of suitable size will serve. The front should be provided with two openings, one arranged to mount a standard size safelight in a sliding frame and above it a ground-glass viewing screen which can be permanently mounted. Over the latter an opaque metal cover in a sliding frame is mounted, to keep out stray light from the box when in position. For viewing, the metal sliding cover is partially removed, thus serving to examine a negative or lantern slide after fixation. The inside of the box is painted white, and a 15-watt lamp is mounted inside, opposite the ground-glass screen. An additional refinement is a hinged shield over the safelight which can be opened or closed to any desired degree.

For the printing processes (enlarging papers excepted) one of the best lights is an amber-tinted bulb. Low-wattage bulbs, available in red, are suitable for developing slow non-color-sensitive plates. When these are used, they should be the colored-glass type rather than

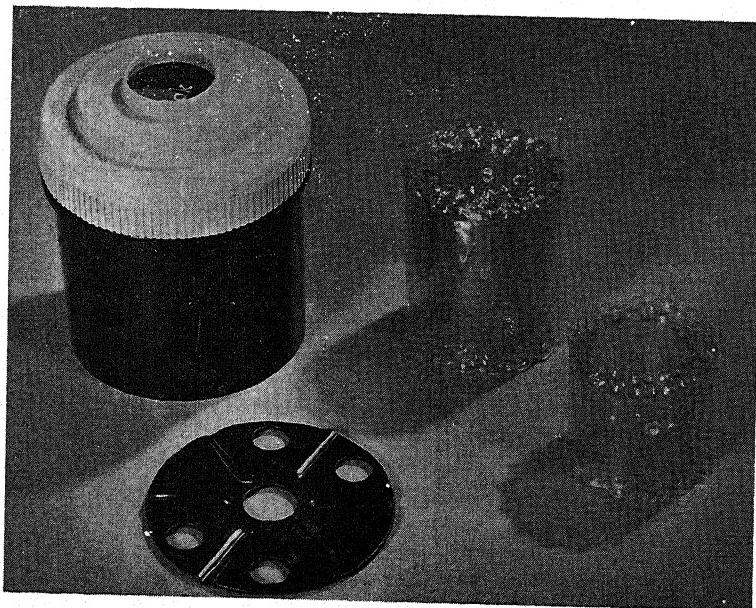


FIG. 220. Kodak Roll-Film Tank

merely lacquer-dipped, since the latter deteriorate rapidly and may soon reach a stage where they are no longer safe for the purpose intended.

The type of equipment required for developing depends largely on whether plates, roll film, or cut film are used. For the former, trays (Figure 217) are preferable, since individual handling of one exposure at a time offers a better method of control. Plates are seldom exposed in such quantities as to warrant multiple development in a tank. Several trays are necessary; it will prove economical in the long run to have the proper sizes for accommodating each size of plate (or printing paper). They are available in glass, porcelain-lined steel, stainless steel, rubber, and composition. The rubber and composition are by far the least satisfactory.

Tanks are desirable for cut film, to eliminate danger of scratching the film. The films are placed in hangers (Figure 218) and allowed to remain in them through the entire process of developing, fixing, and washing. Several small tanks (Figure 219), capable of accommodating the largest film used, will be required. Roll film is best handled in a developing tank of the type illustrated in Figure 220.

Other accessories desirable for the development processes are: scales for weighing chemicals when mixing various solutions; an immersion thermometer, one type of which is shown in Figure 221, for determining the temperature of the developer; and an interval-timing clock, for timing the development. Such a clock sounds an alarm at a predetermined time, which is set to assure proper development for the given combination of plate, developer, temperature, and subject.

There are several such clocks on the market. The General Electric X-Ray Timer is one that can be recommended. A signaling timer is imperative for developing plates and films which must be processed in complete darkness.

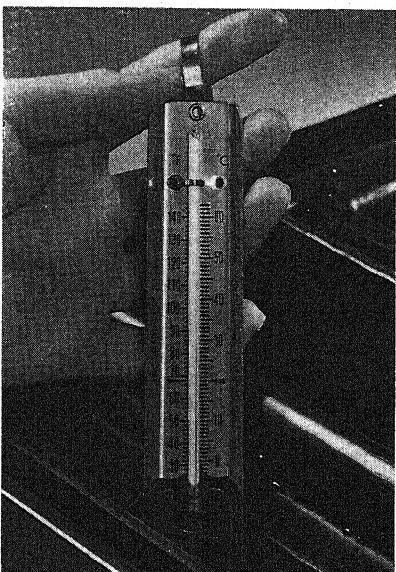


FIG. 221. Kodak Tank and Tray Thermometer

Trays are required for the printing processes, but if trays are used for negative development a duplicate set is seldom necessary. There is considerable latitude in the type of equipment used for printing the positive from the negative. The simplest device is the

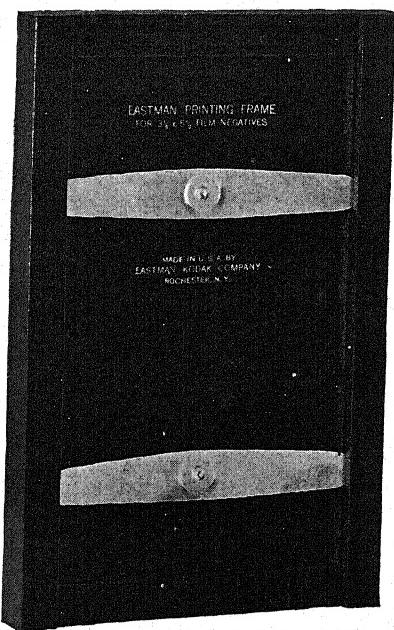


FIG. 222. Printing Frame

graphs may be called upon to furnish enlargements from occasional negatives. For this purpose a machine such as the large Kodak Autofocus Enlarger Model E (Figure 225) is ideal. It will accommodate plates up to 5" x 7".

Printing and enlarging necessitate the use of a suitable timing clock capable of indicating exposure times to a second or even less. That suggested for use in making the exposure of the negative (Figure 83, page 137) is the preferred type.

Plates are washed after fixation in either tanks or individual trays,

* The author has used one of these for many years and has found it a very versatile printer. It is probably because of the modern tendency to use smaller negatives which require enlargement that the demand for this equipment has fallen off to a point where it is no longer a commercial proposition.

printing frame shown in Figure 222. These are entirely satisfactory but slow in operation, and therefore where time is a factor hand printers are desirable. For small negatives the Kodak Photo-Hobby Printer shown in Figure 223 is ideal. For larger-size negatives, commercial hand printers up to 8" x 10" are available. Eastman no longer make the Professional Printer.* Small-size films now in common use have heightened the importance of enlargers for making prints. Many makes of these are on the market. The Kodak Flurolite Enlarger A, shown in Figure 224, accommodates negatives up to 2 $\frac{1}{4}$ " x 3 $\frac{1}{4}$ ". Enlargers of other makes are available to take larger sizes.

Even the photomicrographer equipped to take large-size micro-

and must then be set in a negative rack for drying; therefore at least one drying rack is necessary. Where a number of lantern slides are printed at a time, several racks may be required.

Washing of paper prints necessitates some form of washing device to keep the prints continually agitated in running water. For a few prints only the syphon washers, such as shown in Fig-
in combination with a large tray. The Whirlpool washers (Figure 227) will accommodate a larger quantity of prints. Where many prints must be washed at a time, as in commercial work, the electrically driven rotary washers, such as shown in Figure 228, are ideal. For strictly photomicrographic work, however, the use of large washers is usually restricted to research laboratories doing a considerable amount of work.

Practically all photomicrographs are finished up on glossy paper, squeegeed to give the highest possible luster. This means that ferro-type plates must be available, and also a squeegee roller. Two types of plates are available, black-japanned iron and chromium-plated brass. The latter are better but more expensive. When only a few prints are made at a time and they are small, a hand roller (Figure 229) is adequate. Better results are obtained, however, especially with double-weight paper and large prints, when a wringer is used. Wringers (Figure 230) may be either hand or power operated. An ordinary clothes wringer will suffice.

A print trimmer (Figure 231) will complete the mechanical equipment required for the photomicrographic darkroom, although various other labor-saving devices will be found useful as one becomes more proficient in the work or takes it up more seriously. Among these might be mentioned a series of mask-cutting templates and a circle-cutting tool for use with the templates. The tool is available commercially, but the templates have to be specially made. They are made of about $\frac{1}{16}$ " sheet steel or brass, with circular holes cut in them of the sizes desired for masking the negative in making the prints.

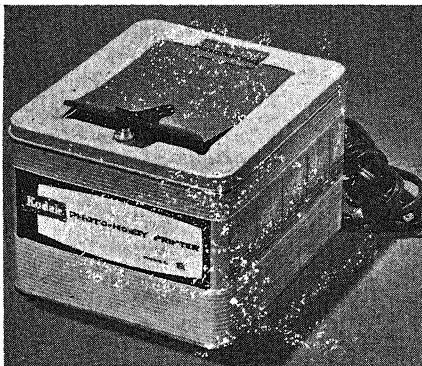


FIG. 223. Kodak Photo-Hobby Printer

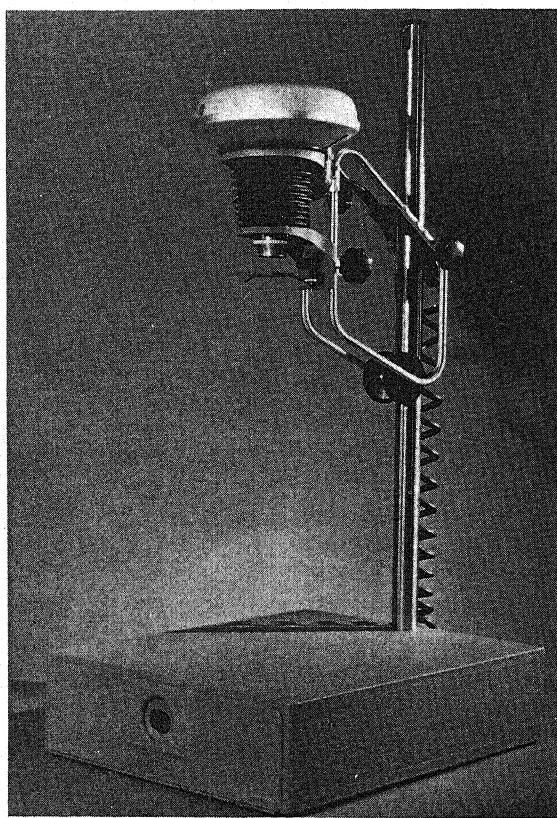


FIG. 224. Kodak Fluorolite Enlarger A

Photomicrographs are preferably printed circular to give the same appearance one obtains with visual observation.

Another useful device is a retouching stand, for spotting defects in negatives and occasional blocking out of undesirable backgrounds.

(3) Glassware

Darkroom glassware requirements are not extensive. The most important pieces include graduates — preferably two-ounce, sixteen-ounce, and thirty-two ounce; a glass funnel (16-ounce size) for filtering solutions; stirring rods; and a collection of bottles for the various solutions it may be convenient to keep on hand. For storing con-

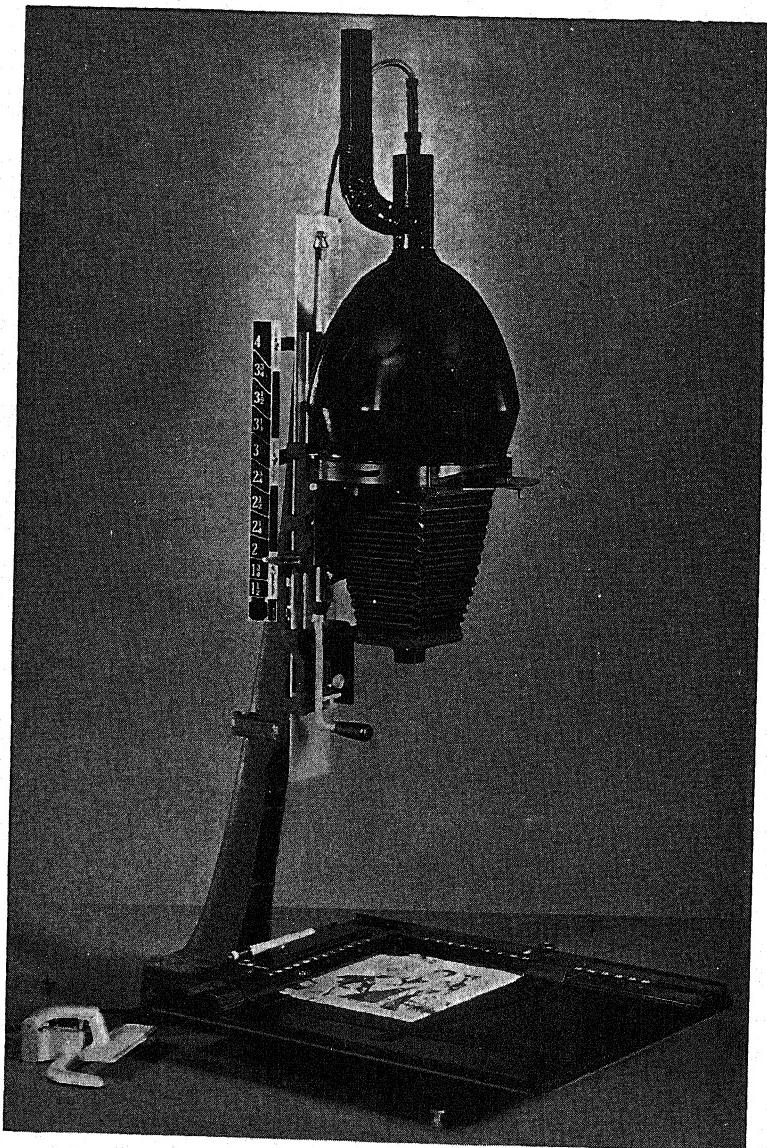


FIG. 225. Kodak Auto-Focus Enlarger

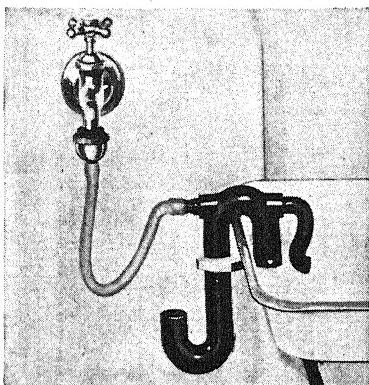


FIG. 226. Kodak Automatic Tray Syphon

is done with prepared developers, especially of the individual glass-tube type, and prepared acid hypo is used, these items can constitute the entire requirements. Probably, however, no amateur photographer will long be satisfied with such limited facilities. Also, the cost of ready-made solutions is relatively high.

centrated developing solutions, the bottles employed should be of such sizes that the solutions completely fill them, thus excluding any appreciable quantity of air and so preventing oxidation. Their keeping qualities are greatly enhanced thereby.

(4) Chemicals

For the amateur interested only in an occasional photomicrograph, the list of chemicals required is not extensive. When all development

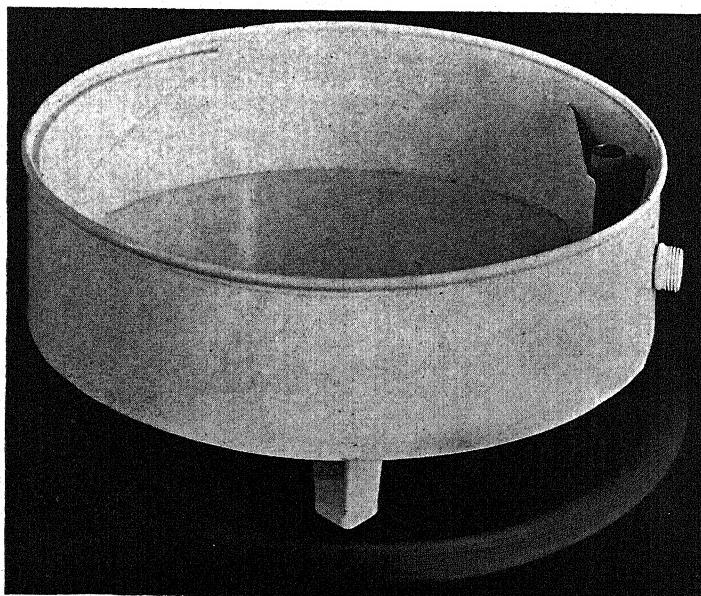


FIG. 227. Whirlpool Washer

The developing agents required will naturally be governed by the specific type of developer standardized upon, especially for negative development. These may include such chemicals as "pyro" (*pyrogallol*), *glycin*, *rodinal*, and other recommended developers. For the beginner, however, the following should suffice:

Metol. Sold by different manufacturers under various trade names such as *Elon*, *Rodol*, *Photon*, *Pictol*, etc.; chemically known by the somewhat high-sounding name of monomethylparaminophenol. This is a soft developer, and is usually employed in combination with hydroquinone for both negatives and prints under the general designation of "M-Q developer."

Hydroquinone. A more contrasty developer, commonly used in combination with metol. By variations in the percentages of metol, hydroquinone, sulfite, carbonate, and bromide, almost any desired degree of contrast can be produced.

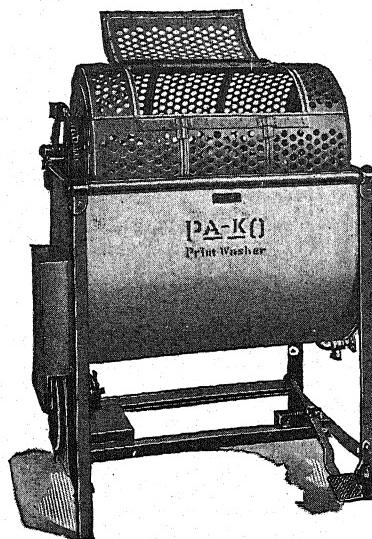


FIG. 228. Pa-ko Large Rotary Print Washer

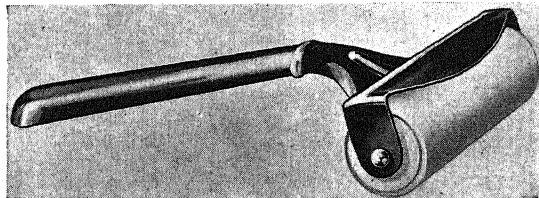


FIG. 229. Hand Roller for Squeegeeing Prints

Amidol (Diaminophenol). A very soft-working developer, useful for bromide paper but also good for soft prints from harsh negatives. It does not require that an alkali be used with it, and hence has another valuable property seldom appreciated. As it will stand a large amount of restrainer (bromide), old printing paper, useless with ordinary developers, can often be made to yield beautiful whites without a trace of fog.

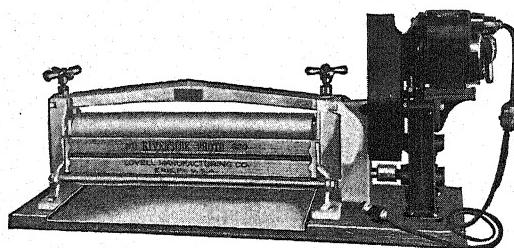


FIG. 230. Ferrotype Plate Wringer

Fine-grain developers. Workers with miniature cameras, with which considerable enlargement of the negative is required for producing a satisfactory print, will do better at the start to employ one of the many commercial fine-grain developers on the market for this

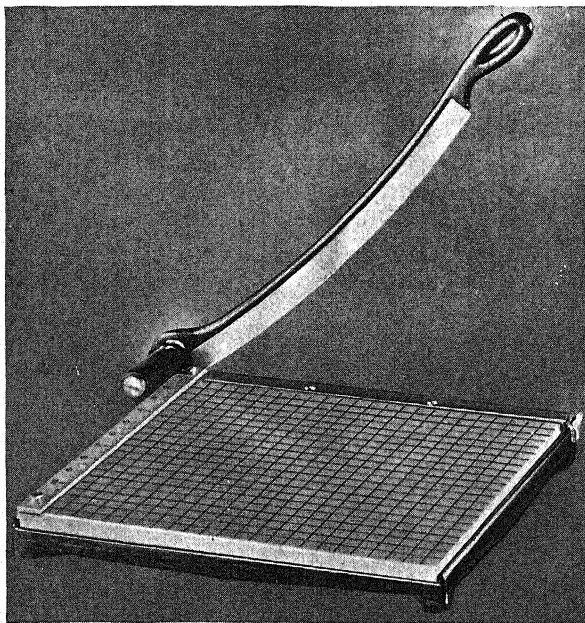


FIG. 231. Print Trimmer

purpose. These come ready prepared and give superior results for this class of work.

In addition to the developing agents, the following chemicals should be available:

Hypo	Potassium alum
Sodium carbonate	Borax
Sodium sulfite	Sodium bisulfite, or
Potassium bromide	Potassium meta-bisulfite
Acetic acid	Potassium ferricyanide

These will be found adequate at the start and can be added to later as the need may arise. Among the more important additional chemicals of value in ordinary photographic work might be mentioned:

Potassium chrome alum
Potassium dichromate
Potassium permanganate
Formalin - (40% formaldehyde)
Sulfuric acid

Recent years have witnessed the introduction of many improved developers, fixing-agent formulas, and modern methods of packaging and merchandising chemicals. The latter can now be purchased in quantities and formulas to suit every user of them. Figure 232



FIG. 232. Some Kodak Developers and Their Packaging

illustrates a few kinds of Kodak developers and the different manner in which they are packaged by the Eastman Kodak Company. Serious-minded photomicrographers should keep abreast of new formulas as they are introduced and determine their value for their own needs.

Developing and Printing Technique

Appreciation of certain underlying principles involved in the developing of negatives and prints will enable one often to detect reasons for poor results and frequently to improve the quality of the work. As we have said, amount of contrast can be controlled by use of different developers. The exposure of the negative also plays an important part in the nature of the finished print. A third factor is the extent of the development of the latent image. For any given combination of developing chemicals, the latent image is gradually built up at a time rate dependent upon the concentration or strength of the solution and the temperature at which it is being used. Cold solutions work more slowly and warm solutions more rapidly. The ideal temperature for development is around 68° F. Below 55° F. the action is very slow; some developers will not act at all at temperatures much lower than this. On the other hand, temperatures in excess of 75° F. tend to soften the emulsion to such an extent that a negative may be ruined unless special precautions are taken. Other unsatisfactory conditions may also result from high temperatures. Between the ranges of 55° and 75° substantially equivalent results can be obtained with most developers by varying the time of development to suit the temperature, provided that the development is carried on to substantially the ideal density. Whatever variations do occur under these conditions are largely the result of changes in the relative action of two developing agents in the solution, such as metol and hydroquinone, at the various temperatures. Metol, for instance, continues working at 55° F., while hydroquinone becomes practically inert at around 60° F. The effect in M-Q combinations is therefore somewhat similar to a change in the percentage of the two active agents in the developer.

Weaker developers must be allowed to act for longer times to produce equivalent density in the negative. In general, however, strong solutions yield greater contrast, while weaker solutions tend to decrease the contrast, thus producing softer negatives. Weak developers may be weak because of greater dilution in the beginning, or a strong developer may become weak through exhaustion resulting from repeated use. Also, developers which have been mixed up for some time, as stock solutions, may lose so much strength through oxidation that results are not identical over an extended period. All of these factors should be always borne in mind.

Agitation of the developer during development hastens the action. With tray development this is easily accomplished by gently rocking the tray. This is one reason for frequently observed superior results with plates, since the latter are more easily handled by tray development, especially when, as in photomicrography, they are developed one at a time.

The particular type of plate employed has an appreciable effect on the development to which it should be subjected. With any given plate there is a range within which the density produced by uniform development conditions is directly proportional to the logarithm of the exposure. This is usually portrayed graphically as the D-log E curve (Figure 233).

The proportion of the curve which is a straight line will vary with different kinds of plates. The region of correct exposure is the

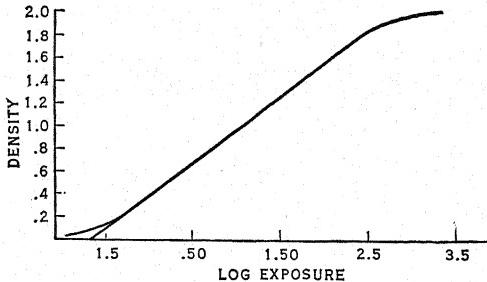


FIG. 233. Exposure Curve. D-Log E Curve

straight-line portion of the curve — that is, the various gradations of tone within the object should, if possible, all fall on the straight line. In photomicrography by transmitted light, where the background is often extremely bright as compared with the object itself, the exposure of the background will, with many types of plates, fall on the top curved portion of the curve. That is, it will be overexposed, but this is not objectionable. It is the tonal range of the object portrayed which should be kept within the straight-line portion of the curve wherever possible.

Since there is in development, at the exact moment of beginning of darkening, a zero difference in the actual amount of contrast in the negative, between those portions which have received the maximum amount of light and those exposed to the minimum light, the function of the developer as regards the time factor is gradually to build up the

difference in density of the various parts of the image. This difference is designated the *development factor* or *gamma* (γ). It is shown in its relation to the slope of the density curve in Figure 234. Study of this graph will reveal that the *ratios* of the straight portions as regards the densities are the same in both fractional development curves, but the *actual differences* are greater as the value of gamma increases. In other words, the steepness of the curve increases with increase in gamma, indicating an increase in the contrast of the negative. This

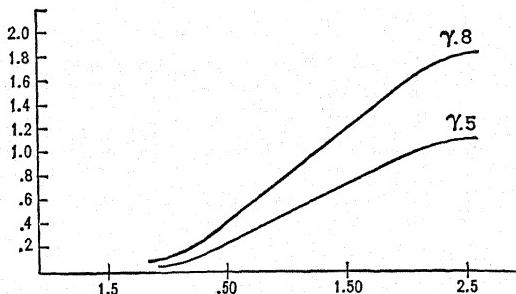


FIG. 234. Graph of Development Time

contrast continues to build up to a maximum designated *gamma infinity* (γ^∞), after which further development causes a decrease in the contrast. Development beyond γ^∞ is called *overdevelopment*. Full development is usually considered to be reached at around $0.8 \gamma^\infty$.

The actual value of gamma infinity is determined by the type of emulsion. A soft plate has a low value. Usually this type of emulsion is relatively fast. The slow process type of plate possesses a high gamma infinity. In order to reduce the actual contrast normally present in such plates, it will be apparent from Figure 234 that, assuming a full exposure has been given, reduced contrast to almost any degree can be obtained by giving a shorter development. In doing this it is often useful to employ weaker developing solution so as to provide a greater latitude in the development time. The desired gradations in the negative are more easily secured with slower development.

From the practical standpoint, most developer formulas have the time of development at a given temperature stated so as to yield an approximation of $0.8 \gamma^\infty$ for average types of plates. Some makes of plates (e.g., Wratten M and Wratten Panchromatic) have cards packed with each box giving the proper development time for low,

normal, and high contrast for the emulsion number of the particular box, with the recommended developers. This is a valuable aid toward securing ideal results, since the development can be done on an exact time and temperature basis, even in total darkness.

Some workers like to observe the density of the negative by holding it up to the safelight for examination by transmitted light. This is a poor practice, since fogging can easily occur. A safer method, if an examination must be made, is to observe the image build-up on the back of the plate. When the high lights (i.e., the most dense portions) begin to show through, it is an indication that development has taken place through the entire depth of the emulsion in these portions and there is little chance of extensive underdevelopment. Another method is to observe a decided darkening of the shadows (i.e., those portions receiving the least light) on the front of the plate before considering development to be complete. Of course, variations in individual subjects considerably vary the application of either of these methods for completely satisfactory results; also, the emulsion of the plate itself is a factor. Experience, however, soon establishes the standards to go by. When experience is gained by repeated observations of the appearance of negatives under the time and temperature method, correlated with the final results, it is a big asset.

Should extremely dense or extremely thin negatives consistently result in spite of faithful application of the time and temperature method, the trouble almost always can be traced to over- or underexposure. To correct such a fault, bear in mind that the effect of the exposure time on density varies in geometric ratio, hence the first steps should be taken by halving or doubling the exposure time, as may be necessary. If the correction is still insufficient, the new trial time must be again halved or doubled.

When development is completed, the plate (or film) should be rinsed in water acidified with acetic acid (see formula at end of chapter) to stop development and neutralize the alkali of the developer so that the hypo solution will not be contaminated with it. A few seconds' rinse usually suffices. The negative is then placed in the hypo bath. It should not be examined by white light until the milky appearance of the emulsion has completely disappeared. The total time in the hypo should be about twice that required for the apparent removal of the unaffected silver salts (i.e., the milky appearance), in order to assure complete fixation. The negative can then be washed in running water until all the hypo is eliminated. If running water

from a tap is allowed to flow directly over the negative, ten to fifteen minutes is ample, but when the negative is in a tray or container where a considerable body of water stands, the time should be at least one-half hour. When running water is not available, it should be washed in about a dozen changes of water in a tray, over a period of about an hour. After washing, the negative should be lightly rubbed over with a tuft of wet absorbent cotton, rinsed, and set aside in a negative rack or in hanging clips to dry.

Paper positives from negatives are made by using the same basic principles as for negatives. Photomicrography does not call for the superabundance of effects, printing processes, types of paper, etc., usually employed by the professional or amateur photographer, since practically all prints are made on white glossy paper, squeegeed to give the highest possible gloss.

The paper stock is furnished in thin, or single-weight, and heavy, or double-weight paper. The latter, though more expensive, is preferable, and after being once used will probably never be abandoned for single-weight prints, for photomicrographic purposes. The majority of photographic paper manufacturers furnish glossy paper in as many as five or six degrees of contrast, usually designated by numbers.

In the Azo (Eastman) and Apex (Defender), two of the most commonly used papers, #2 is considered the proper grade for normal negatives and the expert photomicrographer should expect this paper to suffice for at least ninety per cent of his work. Number 0 is very soft, for use with extremely contrasty negatives, while #5 is an extreme-contrast paper, suitable for negatives devoid of contrast.

The intermediate grades enable one to work slightly on the soft or contrasty side, as may be desired for individual negatives. The degree of contrast can also be controlled to some degree by the developer employed, the exposure and time of development, just as in the case of the negative. With normal negatives, the best results are obtained with a soft, slow-acting developer, rather than a contrasty one.

The exposure time for a given negative and constant light source will be found to vary greatly with the different grades of paper, #0 being the fastest and #5 the slowest. There are some differences in the speeds of the same contrast grade of paper as made by different manufacturers, and, also, the degree of contrast for a given number may vary somewhat. Because of these conditions, it is desirable either to standardize on one make of paper, or establish comparative refer-

ence data as to contrasts, printing times, etc., for different brands so that recorded printing data for a given negative can be translated in terms of any of the papers likely to be employed.

Printing may be done in either of two ways — by contact or by projection. In contact printing the emulsion surface of the paper is placed directly against the emulsion side of the plate or film, and must be held in contact under pressure while the print is being exposed. The simplest apparatus provided for this purpose is the printing frame (shown in Figure 222). It should be needless to remark that loading of the printing frame should be done only in the presence of a light

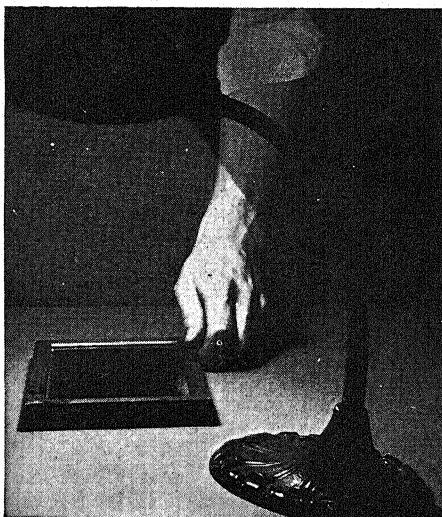


FIG. 235. Exposing Print in Printing Frame

which is not capable of fogging the paper, and that care must be taken to have every bit of sensitive paper (such as the box or package of unexposed paper; prints already exposed, but not developed; and prints in the developer undergoing development) thoroughly covered before turning on the light to expose the print.

When printing is done in a frame, in making the exposure the distance between the lamp and the printing frame should be maintained at a constant, fixed distance. This can be done as illustrated in Figure 235. The frame should be held at right angles to the central beam from the lamp and at the same time should be sufficiently far away that the light paths to the outermost corners of the print are not

appreciably more than the distance to the center of the print. Otherwise uneven illumination will result. This means that a large picture must be held farther away than a small one to secure even illumination over the entire area of the print.

As the light intensity varies inversely as the square of the distance from the light to the negative, when the printing frame is moved twice the distance away, the exposure must be increased by four times for an equivalent exposure.

In order to produce a circular picture surrounded by a white border, a circular mask of the desired size, cut from thin black paper, should be placed between the negative and paper, in the printing frame. Circular prints are usually trimmed square after finishing, with a white border of from one-quarter to one-half inch margin of white on the sides. This allows the cutting of test exposure strips from the long dimension of the paper. For instance, if the paper size is 5" x 7", the maximum square would be 5", the circular picture about $4\frac{1}{2}$ " in diameter. A 1 $\frac{1}{2}$ " strip can be cut with safety from the 7" dimension of the paper, to provide a test strip for determining the proper exposure time.

Negative envelopes are available for storage and protection of the negatives. These provide an ideal place on which to record the printing data. The make and grade of paper and exposure time should be recorded, as well as the developer, if different ones are employed which require a variation in the exposure time.

A small printing machine (or box), such as is illustrated in Figure 223, is a great convenience, not only because of the increased speed of operation, but because the printing light is completely enclosed. There is no need for covering up the box of paper or other sensitive material every time a print is exposed, as must be done with printing frames.

Printing by projection is desirable only when the size of the finished print is required to be larger than the negative. Such printing calls for either a projection printer or an enlarging machine. Where the enlargement exceeds a couple of diameters, the ordinary contact paper is not usually satisfactory because of the inefficiency of the illumination systems employed, and hence bromide enlarging papers must be used. These are extremely fast as compared to contact printing papers, and therefore must be handled in more subdued light, such as the safelight #0. Bromide papers call for weaker developers than contact papers, and the development time is longer.

Whatever paper is used, it is preferable to employ with it one of the specific developers recommended by the manufacturers.

Prints should be rinsed in an acid short-stop bath before being placed in the fixing bath. The latter should be clear and fresh in order to assure freedom from staining, and complete fixation. As paper prints do not show any change in the fixing process (so apparent in the case of the negatives), the only way to be sure of perfect fixation, without which the prints would not be permanent, is to rely on the solution being of suitable strength and acting for a sufficient time. If unused pieces of old plates are saved, the strength of a hypo solution can be tested by observing the rapidity with which the milky emulsion clears when placed in the hypo. If it takes more than a couple of minutes for a marked change to occur, the solution should be thrown away and a fresh one used. Ordinarily about fifteen minutes in the hypo is ample for prints. They should be well rinsed on removal from the hypo bath and, when the batch is completed, washed in running water for about an hour.

The next operation is the placing of the prints on the ferrotype plates, face down, directly out of the water, without draining. Chromium-plated plates rarely require preparation other than thorough washing in plain water, but the japanned plates should be waxed before being used, and at any subsequent time when evidence of sticking appears. Consult the formulas at the end of the chapter for the wax solution and method of applying it. The ferrotype plate should be rinsed in cold water before being used, and as much water as it will retain be allowed to stand on it.

When the print is on the ferrotype plate, it must be squeegeed into perfect contact, either by running through a wringer or with the hand roller; then all surplus water must be wiped off and the plate set aside for the print to dry. When only partially dry it will adhere tightly to the plate as though glued to it, but when completely dry should fall off of its own accord. If it does not, a little loosening at one edge will enable it to be easily stripped from the plate, if the waxing has been properly done.

It then remains only to trim the edges of the print to the proper size and the job is done.

An added convenience for the future identification of the print is a rubber stamp for use on the back of the print, when the photomicrographer's (or company's) name, and space for the negative number, subject of picture, and magnification.

Reduction and Intensification

When a negative is too dense to allow a satisfactory print to be made from it, or, on the other hand, is thin and lacking in density, it is sometimes possible to improve its printing qualities by reduction or intensification. Reduction is a chemical process which dissolves out a portion of the silver deposit in the emulsion so as to make it less dense. Intensification builds up a greater metallic deposit, either of silver or some other element, on that already present, to provide greater density.

Although in general the photomicrographer will find it more advantageous to throw away a negative not up to standard and take the micrograph over, rather than attempt to salvage the poor one, there are occasions when this may not be possible. For this reason familiarity with the methods of reduction and intensification is desirable.

Negatives that are too dense result from either overexposure or overdevelopment, or a combination of both. Since both excessive overexposure and overdevelopment tend to reduce contrast, such negatives are apt to produce flat prints, although this is not always true of negatives benefiting by reduction. In the majority of cases the obvious reason for reduction is an exceedingly dense negative requiring a printing time running into minutes, instead of five or ten seconds for an average-density negative.

On the other hand, cases arise where the object photographed is naturally contrasty in some of its component parts. It should have been photographed on a soft plate, with a full exposure and a development on the short side, but instead, may have been taken on a contrasty plate and received full development. The result is a contrasty negative requiring a long printing time to give detail in the dense portions, while the remainder of the negative yields a fine print with a normal printing time. Partial correction of such a negative through proper reduction may improve it.

No single reducing method will suffice to meet every condition, as three possibilities of improvement exist. For instance, (1) with a negative which yields a print of proper contrast but requires an excessively long time to print, what is needed is proportionate reduction so that while the negative is made less dense, the contrast is not changed; (2) a dense negative producing a flat print requires reduction which at the same time will increase the contrast; while (3) one which is ex-

cessively contrasty must be reduced in such a way that the contrast will be diminished.

Many different reducing formulas have been proposed to meet these conditions, but those given at the end of the chapter, together with their characteristics and methods of use, will be sufficient for the average photomicrographer. As a matter of fact Farmer's reducer will probably be the only one required for ordinary work.

Thin negatives result from either underexposure or underdevelopment. In ordinary photography underexposure may occur from sheer inability to take a picture under conditions where it can be fully exposed. Combinations of available light, emulsion speed, and movement of the object (which determines the speed at which the exposure must be made) are sometimes fixed to such extent that a fully exposed negative cannot be obtained, and a retake under more advantageous circumstances is impossible. The only recourse is to build up a fairly good or passable negative by intensification. In photomicrography one seldom encounters similar conditions. If an underexposure occurs, it is usually the direct result of miscalculating the proper exposure time. Therefore, thin negatives which result from underexposure should be taken over rather than corrected by intensification.

The possibility of intensification of a negative lies in the ability of the silver deposit in the emulsion to build up further metallic density in substantially the same proportions as already present. In areas where no silver exists in the negative, as usually happens in underexposure, there is nothing to build upon, so that intensification, though it may produce contrast between light and dark portions, will not provide desired detail in the dark areas.

The situation is quite different when thinness of the negative results from underdevelopment of a fully exposed plate. Here there will be silver present in the proper ratios, and if it can be built up by proportionate additions of silver or other metal, just as though development were being continued from the point where it was stopped, a fairly good negative may be produced through intensification. It should be borne in mind, however, that in many cases where intensification *could* be accomplished with profit, the simpler method may be to retake the exposure and develop it properly rather than bother with intensification which may or may not give satisfactory results.

A few formulas and instructions for intensification are included at the end of the chapter for the benefit of those who may desire to try

them, or for those rare cases where it is impossible to retake the negative.

The Making of Lantern Slides

Because of the scientific and educational value of photomicrographs, the photomicrographer is frequently called upon to produce lantern slides from some of his negatives. Apart from a few additional steps in the process, this class of work does not differ fundamentally from the making of ordinary negatives and prints.

The standard American size for lantern slides is $3\frac{1}{4}'' \times 4''$ (the longer dimension being the horizontal one as the slides mount in the projection lantern).*

The lantern slide is a positive transparency, printed on glass instead of on paper. Manufacturers supply lantern-slide plates of the standard size. They are very fine-grained slow emulsions and can be had in at least three degrees of contrast — soft, medium, and contrasty. For properly timed and developed negatives, the medium or normal plates are the best.

The picture size, to utilize the most of the plate area, should be about $2\frac{3}{4}''$ diameter for a circular print, or $2\frac{3}{4}'' \times 3''$ for a rectangular one. In unusual instances these dimensions can be increased slightly, and of course they can be as much under as desired, but at the expense of projected picture size when full-size pictures are also shown on the same occasion.

There are three possible methods of printing lantern slides, depending upon the size of the negative and the area to be embraced in the lantern-slide picture. The simplest case is where the lantern-slide print is to be the same size as the negative, since this can be accomplished by contact printing. This is preferably done in a printing frame; the intensity of the light in a printing machine set up for gas-light paper is usually far too great to allow of proper control of the exposure. Generally about one to three seconds' exposure through a normal negative 3 feet from a 10-watt lamp suffices. The negative need not necessarily be the same size as the lantern slide for contact printing. If small prints are satisfactory for projection, films from minicams can be made into standard-size lantern slides by contact

* The standard English size, also quite commonly used in Canada, is $3\frac{1}{4}'' \times 3\frac{1}{4}''$. Manufacturers of lantern slides supply holders which will accommodate either size, and special holders are made which will take both indiscriminately.

printing. Care must be taken, from the aesthetic point of view, that the film be properly centered and aligned on the lantern-slide plate. Should the negative be at all dense, the use of a black paper mask around the negative is desirable, not primarily to stop the blackening of the plate around the picture, but to obviate almost certain halation around the edges.

Lantern slides can be made by contact printing from even the largest plates (e.g., 8" x 10") where only a specific area is required to be shown and can be included within the confines of the lantern-slide picture space.

Usually photomicrographs on minicam film which are to be made into standard-size lantern slides should be enlarged to the proper dimensions. This enlargement can be done easily in any of the standard minicam enlargers, merely substituting the lantern-slide plate for the bromide paper. If an intense light is employed in the printer, suitable for enlarging on gas-light papers, it must be cut down to the bromide paper range to allow sufficient latitude in exposing the plate.

The third condition obtains when photomicrographs on large plates must be reduced to lantern-slide plate dimensions. For this work some form of copying camera must be used and an illumination box provided for uniformly lighting the negative from the back. Often the photomicrographic camera can be utilized for this purpose, or the copying may be done with any camera which will accommodate a $3\frac{1}{4}'' \times 4''$ plate and which possesses a bellows length ample to provide focussing flexibility for the lens employed for copying. For reducing 5" x 7" plates ($4\frac{3}{4}''$ circle) to lantern size ($2\frac{3}{4}''$ circle) a 6-inch lens is satisfactory and the reduction is roughly to three-fifths of the original.

Since the scale of a transparency is not limited to the same extent as a paper print, a more contrasty development is permissible and gives to the projected picture a snap that is pleasing to the eye. Development should be complete or the picture will appear flat. One must not be misled by the appearance of the image while the plate is still in the developer, for fixation changes the values of the tones materially. Development until the picture appears far too dark (if it were a paper print) will be found to give a much better result when the slide is projected. Development, rinsing, fixation, and final washing of lantern slides do not differ from the standard process for plates.

When the plates are dry, the next step is the mounting and binding operation. For this, three additional supplies are necessary. These

are: a mat made from opaque paper of the same size as the lantern slide and with a hole cut in it of the desired size and shape to reveal only the picture area; a lantern-slide cover glass, which is merely a clear glass of the same size as the lantern slide; and a piece of binding tape for passepartouting the glasses together.

Mats can be purchased with various-sized openings, but when only occasional slides are required and the pictures are not such as to be accommodated by standard mats, they can be made of opaque paper as necessary. Circular openings can be scribed with a pencil compass and cut with small scissors, or a circular print cutter can be used. For a large quantity of circular mats a steel punch of the desired size can be obtained. Square and rectangular openings can be cut out with a safety-razor blade by first making a cardboard template of the desired size and shape and cutting around it.

If desired, thumb markers, the photomicrographer's name, and subject of the picture can be typewritten on paper inserts and included within the slide before binding, or they can be pasted on the outside later.

The glass covers are obtainable from any supply house dealing in photographic supplies. These suppliers also carry the binding tape, which comes in two forms, continuous rolls or cut pieces 15" long. The binding is usually one-half inch wide, and gummed on one side. It is applied by starting at one corner and doubling the corners over so that it runs continuously all around the slide. In order to hold the two pieces of glass together while being bound, spring clips mounted on a baseboard are available at small cost.

In assembly of the mat and cover to the positive, care must be taken that the *emulsion* side of the latter is on the inside, since the major purpose of the cover glass is to protect the film against scratching.

The introduction of miniature film in recent years has resulted in the development of small machines suitable for projecting this size of transparency. If photomicrographs are to be made into lantern slides for use in these miniature projectors, the only change in the procedure lies in the size of lantern-slide plates employed (2" x 2") and the greater reduction necessary when any photomicrographic outfit larger than a minicam is used.

With ordinary lantern slides it is important that they be inserted in the projector properly, as otherwise they will be upside down or reversed left and right. To assure proper placing in the lantern, which actually means placing them in inverted, it is customary to place an

indicator, known as the *thumb mark*, on every slide. This can be a dot, circle, star, or even a small square of paper pasted on the outside. It is placed in the lower left-hand corner as one holds the slide before him and views it as it should appear on the screen.

In placing the slide in the machine, the operator grasps the slide in such a manner that the thumb of his right hand is over the thumb mark as he faces the screen, and drops it into the lantern in this position. In other words, it is then in the upper right-hand corner, and hence is inverted, but it is not reversed from left to right as long as the operator is facing the screen, that is, is viewing the picture as if he were holding it in front of him.

With many photomicrographs, it is unimportant how they are viewed, but for the sake of uniform practice, it is desirable that every slide be provided with a thumb mark. In this way a lecturer becomes accustomed to the exact appearance of the picture on the screen and can readily locate a particular object to be pointed out. Then again, there are microscopic objects which should always be shown in the proper position. To reverse them would seem to the expert biologist just as serious as to project the picture of a tree or a horse upside down and attempt to explain it to an appreciative (?) audience.

FORMULAS

The following formulas are provided largely as a ready reference list of those published and recommended by various photographic plate and paper manufacturers. They are by no means the only ones which are in use or known to give satisfactory results, but should be ample to meet the average photomicrographer's requirements.

Since strictly photomicrographic work does not call for toning effects, various processes widely used by the pictorial photographer, and unusual techniques, all formulas pertaining to these have been omitted. In addition, there are many formulas employing special developing agents which are of considerable value in the general photographic field, but unnecessary for photomicrographic purposes, which have been omitted. These can be found, if required, in almost any of the handbooks and more ostentatious works on photography.

If one desires to process color transparencies (since the obsolescence of the older films, Lumière, Agfa, Dufay, etc., leaves only two major color films which are adapted to customer processing, i.e., Kodak Ektachrome and Anscochrome films), complete information

covering these should be secured from the manufacturers' publications covering the processing. Processing of these, while more complicated and time consuming than with the older color films, is continually becoming more common and with practice the results obtained can equal those of commercial laboratories.

Certain procedures and precautions apply in general to the making up of every formula, and hence it is well to understand these at the outset. Among these the following are of importance — sometimes vitally so, and at other times may be considered only as recommended practice; but one will always be safe by assuming them to be factors in every case, not only in formulas given here but those derived from other sources.

(1) The water used for mixing solutions should be pure. Theoretically, this would mean that only distilled water could be used, and hardships often result from such a requirement. Actually, most tap water can be used, especially if it is filtered to remove iron or suspended matter when present, and when boiled and filtered (on cooling) if the water is that type known as "hard." Hard water can be recognized by its tendency to neutralize the effect of soap on the hands. Calcium and magnesium salts are present in such water and can cause much trouble. Boiling tends to precipitate them. With these exceptions, good clear tap water should suffice; however, if trouble is experienced, look carefully to the water used.

(2) Only chemicals known to be chemically pure or prepared by one of the chemical companies especially engaged in the manufacture of photographic supplies, should be used. In some cases the same chemical (e.g., metol) is known by a specific trade name with each manufacturer. Therefore, in any formula calling for such a substance by one name, it must be understood that the product of another supplier can be substituted with safety, if one first makes sure of the actual identity of the chemical involved.

(3) In making up a formula of any kind, *always* follow the order in which the chemicals are given, unless specific instructions are added as to the method of mixing. Sometimes this is very important, as the presence of one constituent is necessary to prevent others from reacting with each other, or a reaction may occur which is dangerous.

(4) Some chemicals (e.g., sodium carbonate) may be supplied in the crystalline form, that is, having the natural water of crystallization; with this water all removed (anhydrous); or with a part of it removed. The actual strength of the chemical depends upon the

amount of water present, so that one must know which is called for in the formulas and if that he is using is not of the strength specified, an adjustment must be made in the amount used so as to meet the specifications of the formula. How important this is will be apparent when it is appreciated that crystal sodium carbonate is only 37% as strong as anhydrous. Unfortunately, the anhydrous absorbs water by standing, after being opened, to the extent that its strength may decrease to 85% (i.e., the monohydrated form). The monohydrated salt is stable, and hence is the most desirable form to stock. Should a formula call for anhydrous (desiccated) carbonate, the amount should be increased 15% when the monohydrated is used. On the other hand, should a formula specify crystallized carbonate, the amount of monohydrated used should be 43% less. If desiccated (anhydrous) carbonate has been stocked and exposed to the air for some time, it is better to assume it to be not over 90 to 95% of full strength (i.e., which is about 98%) and increase the quantity called for accordingly.

(5) Temperatures up to 125° F. are often recommended for mixing developer solutions; the primary purpose is to effect ready solution of chemicals less soluble in cold water. When mixing concentrated stock solutions it is often necessary to utilize the higher temperature in order to get everything in solution. *But it should never be exceeded* and if time permits, it should be kept lower to be on the safe side. After mixing, a solution should be allowed to cool to normal room temperature before being used.

DEVELOPING SOLUTIONS

It is hardly necessary to go into detail as to the numerous formulas recommended by the various photographic plate, film and paper manufacturers. Some of these have copyrighted names, not only for the complete developing solutions, but for the active developing agent as well. For a fairly complete list of formulas for developing purposes and other valuable developing aids, reference can be made to the Eastman Kodak Company's booklets, *Developing, Printing, Enlarging, with Kodak Materials, Processing Chemicals and Formulas*, and *Photography Through the Microscope*. For those desirous of a more complete library on the subject, there are numerous books available, covering history, theory and practice in the photographic art.

Developing solutions can be prepared as formulated in these books

for the various types of results desired. All manufacturers also publish formulas for use with their products. A few standard formulas of long standing are included here for convenience and ready aid if other information is not at hand. The following are especially recommended for the development of negatives.

METOL-HYDROQUINONE BORAX DEVELOPER (D-76)

For medium contrast, with fine grain

	<i>Avoirdupois</i>	<i>Metric</i>
Water (at about 125° F.)	96 ounces	3.0 liters
Metol	116 grains	8.0 grams
Sodium sulfite (desiccated)	13½ ounces	400.0 grams
Hydroquinone	290 grains	20.0 grams
Borax	116 grains	8.0 grams
Water, to make up to	1 gallon	4.0 liters

This developer is especially fine for panchromatic plates and though intended primarily for tank use, works well in tray development, where the time can be spared for rocking the tray during the rather extended development period.

Use the developer without dilution; for tank development 12 to 15 minutes at 65° F. is required. With tray development and agitation by rocking, 7 to 10 minutes is satisfactory.

METOL-HYDROQUINONE DEVELOPER (D-72)

For average contrast on fast orthochromatic plates

Stock Solution	<i>Avoirdupois</i>	<i>Metric</i>
Water (at about 125° F.)	16 ounces	500 cc.
Metol	45 grains	3.1 grams
Sodium sulfite (desiccated)	1½ ounces	45.0 grams
Hydroquinone	175 grains	12.2 grams
Sodium carbonate (desiccated)	2¼ ounces	67.5 grams
Potassium bromide	27 grains	1.9 grams
Water, to make up to	32 ounces	1.0 liter

For average contrast use one part of stock solution to one of water and develop 4 to 5 minutes at 70° F. When maximum density is required use the stock solution full strength.

D-72 is a good all-around universal developer for plates, films, lantern

slides, and paper, when used in the proper concentration for each. For paper prints with cold tones use diluted one to one, and for warmer tones, one of developer stock solution to two of water.

For warm tones on lantern slides, Eastman recommend with their plates as follows:

Soft Lantern plates. — 1 part stock solution to 4 of water; develop 2 to 3 minutes at 70° F.

Medium Lantern plates. — 1 part stock solution to 2 parts of water; develop 1 to 2 minutes at 70° F.

Contrast Lantern plates. — 1 part stock solution to 1 part of water; develop 3 to 5 minutes at 70° F.

CONTRAST DEVELOPER (D-11)

For strong contrast on plates and lantern slides

	<i>Avoirdupois</i>	<i>Metric</i>
Water (at about 125° F.)	16 ounces	500.0 cc.
Metol	14 grains	1.0 grams
Sodium sulfite (desiccated)	2½ ounces	75.0 grams
Hydroquinone	130 grains	9.0 grams
Sodium carbonate (desiccated)	360 grains	25.0 grams
Potassium bromide	70 grains	1.0 liter
Water, to make up to	32 ounces	5.0 grams

Use without dilution for either tank or tray development, when maximum contrast is desired. 3 to 4 minutes is usually ample in an agitated tray and 5 minutes in a tank. When less contrast is required dilute one to one.

DEVELOPERS FOR PAPER PRINTS

Although for paper D-72 can be used satisfactorily, warmer tones and better scale values will result from the following:

PRINT DEVELOPER FOR WARM TONES (D-52)

Stock Solution	<i>Avoirdupois</i>	<i>Metric</i>
Water (at about 125° F.)	16 ounces	500.0 cc.
Metol	22 grains	1.5 grams
Sodium sulfite (desiccated)	¾ ounce	22.5 grams
Hydroquinone	90 grains	6.3 grams
Sodium carbonate (desiccated)	½ ounce	15.0 grams
Potassium bromide	22 grains	1.5 grams
Water, to make up to	32 ounces	1.0 liter

For use, dilute one to one and develop for about $1\frac{1}{2}$ minutes at 70°F .

DEVELOPER FOR BROMIDE ENLARGING PAPERS (55-D)
As recommended by Defender Co.

Stock Solution	Avoirdupois	Metric
Water	32 ounces	1.0 liter
Metol	36 grains	2.4 grams
Sodium sulfite (desiccated)	$1\frac{1}{4}$ ounces	36.0 grams
Hydroquinone	144 grains	10.0 grams
Sodium carbonate (desiccated)	$1\frac{1}{4}$ ounces	36.0 grams
Potassium bromide*	60-144 grains	4-13.0 grams

* The liberal use of potassium bromide even in excess of the quantity called for, is recommended.

For use, take one part of stock solution and add two parts of water. Develop for from $1\frac{1}{2}$ to 3 minutes. Short development makes for warm tones, the longer development for cold tones.

AMIDOL DEVELOPER
For prints, bromide enlargements, and lantern slides

	Avoirdupois	Metric
Water	16 ounces	500 cc.
Sodium sulfite (desiccated)	1 ounce	30 grams
Amidol	45 grains	3 grams
Potassium bromide	15 grains	1 gram

Amidol developers must be mixed up just before using and should be used the same day. The proportions can vary over a considerable range for different degrees of contrast. Increase of the sulfite and bromide at the expense of the amidol tends toward further softness; increase of the amidol percentage moves the effect in the other direction.

FIXING SOLUTIONS

PLAIN HYPO SOLUTION

For fixation of lantern slides which are to be hand colored with water color tints.

Hypo	1 pound
Water, to make up to	64 ounces

For acid hypo to be used for general fixation, add to above from $\frac{1}{8}$ to $\frac{1}{4}$ the quantity of the following stock solution, depending on the amount of hardening desired. In hot weather the stronger solution will be required, but it may not be necessary in cold weather.

Eastman's Kodak Hardener F-5a is especially recommended as a stock solution. It can be obtained in package form. The formula is as follows, for those who desire to prepare their own.

Water, about 125° F. (50°C.)	20	ounces	600	cc.
Kodak sodium sulfate, desiccated	2½	ounces	75	grams
Kodak acetic acid, 28%	7½	ounces	235	cc.
Kodak boric acid, crystals	1¼	ounces	37.5	grams
Kodak potassium alum	2½	ounces	75	grams
Cold water to make	32	ounces	1.0	liter

In case the acetic acid is stocked as glacial acetic acid, to make 28% acid dilute 3 parts of it with 8 parts of water. Dissolve the chemicals in the order given. Add 1 part of the cool stock hardener solution slowly to 4 parts of cool hypo solution (2½ pounds per gallon of solution), while stirring the hypo rapidly.

SHORT-STOP RINSING SOLUTION

Water	1	quart
Acetic acid (28%),	1½	ounce (or ounce glacial)

Use for rinsing plates, films, and paper prints between the developing and fixing processes. It not only stops development but neutralizes a part of the alkali of the developer, which would otherwise be carried over into the hypo solution and cause discoloration of the latter, through oxidation.

REDUCING SOLUTIONS

For Plates and Films

The most useful reducing solution for the photomicrographer and that which may serve all practical purposes for him is:

FARMER'S REDUCER

Stock Solution A

One to two ounces of potassium ferricyanide in 16 oz. water

Stock Solution B

Ordinary plain hypo solution, 16 oz. of hypo to 64 oz. water

This reducer is a surface cutting or subtractive one, and hence will increase the contrast as it reduces. The action is not strictly confined to this result, however, as by a change in the concentration it may become more of a proportionate reducer and not yield so much increase in contrast. The greatest contrast is obtained with a relatively strong solution of the ferricyanide. For use, about one ounce of hypo solution should be added to seven ounces of water and a little of Solution A added to this, just enough to give a yellow color to the hypo solution. This may be seen better if it is added in a white porcelain-lined tray. If the reduction is to be made on a wet negative, thorough washing out of the acid hypo should be done first. If the negative is already washed and dried, it should be wet thoroughly in water before placing in the reducer. The tray should be rocked slightly and the negative examined frequently until the desired density is secured. It must then be rinsed under running water for a few seconds to remove most of the reducer or reduction will continue beyond that desired. This is followed by washing in the usual way as done after the ordinary hypo bath.

This solution does not keep for more than a few minutes after being mixed together, and hence if the desired reduction is not secured after three or four minutes, a fresh bath should be used and the process continued until the desired reduction has been brought about. In most cases, however, when contrasty effects are required, the reduction should have been completed within one minute.

PERMANGANATE REDUCER (R-2)**Stock Solution A**

Water	1 quart
Potassium permanganate	1 $\frac{3}{4}$ ounces

Stock Solution B

Water	1 quart
Sulfuric acid (concentrated)	1 ounce (<i>Pour acid into water</i>)

For use, take 1 part of A, 2 parts of B, and 64 parts of water. When the negative has been sufficiently reduced, place it in plain or acid hypo for a few minutes to remove yellow stain, then wash thoroughly. The only advantage this reducer has over Farmer's is with plates that have been developed in pyro developer and stained excessively in it, since it aids in

removing the brown developer stain. Also, by the use of a very minute quantity of the acid, Solution B, it can be used to remove "dichroic fog" from the surface of a negative, but if the acid be insufficient a staining of the negative from a precipitate of manganese dioxide results. This latter can be removed in a sodium bisulfate solution.

AMMONIUM PERSULFATE REDUCER (R-1)

Water	1	quart
Ammonium persulfate	2	ounces
Sulfuric acid (concentrated)	3.0	cc. ($\frac{3}{4}$ dram)

For use, dilute with two parts of water.

Ammonium persulfate is a super-proportional reducer, and hence reduces contrast as it reduces. This is due to the fact that it acts faster in proportion to the amount of silver present.

It is important that the negative to be reduced be thoroughly washed before reduction, to assure uniform action, and the reduction must be watched carefully since the action ultimately attacks the shadow details and the negative will then be ruined. After reduction and superficial rinsing, the negative must be placed in a solution of sodium sulfite to stop the action and neutralize the persulfate remaining in the emulsion. It should then be washed in water, as usual.

These two types of reducers should be ample for practically every condition that is likely to occur in photomicrography, since slight flatness or contrast can be corrected by the use of the corrective grade of printing paper. However, for the sake of anyone desiring a proportionate reducer, the following formula is included. It will be noticed that this formula is a combination of the permanganate (subtractive) and persulfate (super-proportional) reducers, since no one chemical is known which by itself will give a strictly proportional reduction.

PROPORTIONAL REDUCER (R-5)

Stock Solution A

Water	1	quart
Potassium permanganate	4	grains (0.3 gram)
Sulfuric acid (10% solution)	$\frac{1}{2}$	ounce (16 cc.)

Stock Solution B

Water	3	quarts
Ammonium persulfate	3	ounces

For use, take one part of A to three parts of B. Use same precautions as with other types of reducers. When reduction is secured, wash in a solution of sodium bisulfite, then in water, before drying.

INTENSIFIERS

Although, as pointed out in the text, intensification should not be resorted to in photomicrographic work when it is at all possible to retake the negative, for an extraordinary occasion it may be necessary to intensify a negative, and hence at least one type of intensifier should be available for the purpose. There are many different intensifiers in use, employing silver, mercury, and chromium as the additive metals. Most of these involve at least a two-step process, bleaching and redeveloping. Probably that which is the least complicated to employ and which results in a fair degree of intensification is the following:

MERCURIC-IODINE INTENSIFIER

Mercuric iodide	2	grams
Potassium iodide	2	grams
Hypo	2	grams
Water	100	cc.

Negatives should be well washed before placing in the solution. The action is progressive and should be watched until the desired density has been reached. In order to make the negative permanent, after washing, it should be immersed in a 1% solution of sodium sulfide until the image, when viewed from the back, has been changed from a grayish to a brown-black color.

It is preferable to mix up the small quantity of this intensifier as required, or it can be purchased in prepared form, which is very convenient.

MISCELLANEOUS

The following miscellaneous formulas will be found convenient at times, in connection with various phases of photomicrographic work.

CLEANING SOLUTION FOR TRAYS

Water	1	quart
Potassium bichromate	3	ounces
Sulfuric acid (concentrated)	5	ounces (add slowly to the bichromate solution)

This solution should be kept on hand at all times for the purpose of rinsing out trays and removing the silver discoloration which occurs, especially in the developing tray. A tablespoonful of the solution is usually sufficient. After clearing the tray, it should be rubbed thoroughly with a wet cloth or tuft of cotton, then well rinsed in water.

FERROTYPE SOLUTION

For treatment of japanned plates to prevent sticking

Dissolve a piece of paraffin wax about the size of a small marble in six ounces of benzol or xyol. Keep well corked.

Apply a little to the surface of a plate and rub over until the solvent has evaporated. Then polish thoroughly with a clean soft cloth until all smeariness has been removed. Repeat whenever there appears the least tendency for the plates to show sticking.

RETOUCHING SOLUTION

Dissolve about a teaspoonful of Canada balsam in four ounces of benzol. The exact concentration can be varied to suit the user's individual requirements.

This solution is required only on rare occasions by the photomicrographer, to correct artifacts in a specimen — dirt, torn tissues, etc. — since general retouching is not desired. For use, apply a drop to the area to be corrected and rub over with a tuft of cotton, leaving only enough of the dry solution on the surface to receive the pencil marks.

DESENSITIZING SOLUTION

When it is desired to watch the development of panchromatic plates under a fairly bright light, the plates may be desensitized by a preliminary soaking in a solution of a dye known as Pinakryptol Green. It is used in a strength of one part of the dye to 5000 parts of water. Two minutes' immersion is sufficient. This part of the process must be carried on under the series 3 safelight, or in the case of super-panchromatic emulsions, in absolute darkness. After the treatment, and after the plates have been placed in the developer, a fairly strong light, such as that employed for the development of process plates, can be used. On the whole, however, the photomicrographer will do better if he does not worry about the appearance of the image during development, but relies entirely on the com-

bination of proper exposure, time, and temperature of development, to produce the correct result.

REMOVAL OF STAINS FROM THE HANDS

Users of pyro developers may expect stained fingers if they are allowed to come in contact with the solution; the fingernails are the greatest sufferers. This stain can be fairly well removed by soaking the fingers in a 20% solution of potassium permanganate. This oxidizes the stain but results in what appears to be an even worse stain. This latter is quite easily removed, however, by a second washing in sodium bisulfite solution or in a weak solution of oxalic acid.

Frequently a little developer, not only pyro but M-Q as well, may get on a good white shirt in the darkroom and not be detected until a dark brown stain shows up some time later. This stain remover works equally well for removing such stains, which otherwise will remain in the goods after an indefinite number of washings.

10

Illustrative Photomicrographs

Among the best methods for improving one's technique in any field of endeavor is the study of the work of others. Photomicrography does not differ in this respect from ordinary photography, music, painting, or other arts where personal ability plays a part. The underlying theory back of this practice can be stated in the old-time proverb, "What one fool has done, another can do."

Naturally some lines of work require an inborn genius, without which all the practice and experience that might be crowded into a lifetime would be useless.

In others, latent talent plays but a small part; continued experience is more largely responsible for final results. Photography and photomicrography are among these latter. This is because, in the last analysis, the work is mainly the product of mechanical (including optical) devices, combined with natural or producible objects, and not all of human ability. For this reason, having before one evidence of what is possible provides for the novice, as it were, a mark to shoot at.

It is for this reason that representative photomicrographs depicting many types of objects and numerous variations in technique are included in this chapter. These have all, without exception, been chosen from among the many thousands taken by the author, for one purpose or another, during his past forty years in the work.

Not all those shown are perfect in every respect, nor do they necessarily represent especially beautiful subjects such as one might pick out for a competitive exhibit of photomicrographs. Rather, many of them will seem quite ordinary to specialists in the particular field in which the micrographs would be classified. In only a few instances have pictures been included which possess general interest; the main emphasis has been placed on illustrative examples covering a large number of lines of work. That the selection of the pictures shown has been made with difficulty may be appreciated when it is realized

that, in the realm of haematology alone, the author has taken upwards of a thousand photomicrographs, while in metallurgy the number is many times this figure. Yet these two subjects are represented in the list by a paltry half-dozen.

There will be found examples of low-power work, by both transmitted and incident light, and also of extremely high magnifications, in the region where, according to all theory, "empty magnification" should run riot. Examples of optical sectioning and great depth of focus are also shown.

There are many fields of specialized microscopy not represented in the list. These include such subjects as fluorescence microscopy, chemical microscopy with polarized light, colloidal microscopy, general ultra-violet work, Rheinberg illumination, powdered materials (pigments, activated carbons, fillers, powdered talc, etc.), disease fungi, commercial fibers, and others. Lack of space was responsible for their elimination.

An accurate check of every photomicrograph taken in the country today would probably show a preponderance of them to fall in the realm of pathology; it may seem, therefore, that this field is not proportionately represented in the list. It was felt, however, that the subjects themselves, being so highly specialized, and understandable only to the medical profession, and of so little interest to the average microscopist, did not justify the placing of emphasis upon this type of photomicrograph. The technique involved in this subject does not differ from that called for by histology and other biological sections; therefore, these latter can serve as a guide to the pathological photomicrographic technician.

It is hoped that the method of presenting the pictures with an individual explanation of the subject, special technique, and general problems involved, together with the photographic data, will increase their value as practical examples to workers in various fields and at the same time make them of general interest to those not especially concerned with the specific fields represented by some of them. Some of the pictures may be of interest to biologists even though they may not be interested in photomicrography, because of the unusual features in the objects themselves. Had space permitted, this phase of the subject would have received more attention, as some very unique biological photomicrographs could have been shown, representing rare and little-known conditions.

In the technical data on the pictures, it will be noted that in general

the camera extension has not been given. The primary reason for this is that the figures on the record cards would require recomputing to mean anything to those not using the same type of equipment. The graduation in centimeters on the camera extension bar (the figure recorded) is not necessarily the distance of the plate from the eyepiece. In other words, the distance of the eyepiece from zero would also have to be stated, and this record has not been kept. It should suffice to know the lens combination employed, and the magnification. The magnification specified can be secured only at one particular camera length, should anyone desire to duplicate the conditions. If magnification charts are made for each lens combination, as suggested in Chapter 4, it will be possible for anyone immediately to set his own apparatus at the proper camera length, to reproduce the results. Where it is of importance, to emphasize the fact, mention is made of the part played by employing an unusually long projection distance.

The exposure time cannot always be used as a guide for duplicating results on other equipment, inasmuch as the nature of the light source, the density and stain of the section, or other peculiarities of the object, are not necessarily comprehended from the photograph, and may vary over a wide range under two different sets of conditions.

It will be apparent, from study of the exposure data, that the light source most commonly employed was a 500-watt lamp. This was seldom used direct, as in the so-called Köhler illumination, but as in the author's method described in Chapter 4 (page 218), with two pieces of frosted glass directly in front of the light. The transmission curve of these pieces of glass is shown in Figure 133, indicating substantially 50 per cent reduction in illumination. This fact must be taken into consideration in making comparisons of light intensities.

It has not been possible in all cases to give the exact stain used on the section or specimen but an attempt has been made to give an idea of the general color shown by the slide.

Data regarding the prints have not been included. With very few exceptions, paper of #2 Grade (Normal) of hardness has been used, always in glossy finish. None of the pictures shown are enlargements; in every case they are contact prints from the negatives. It is probably needless to add that some of the finer detail which is clearly visible in the originals must of necessity be lost in a half-tone reproduction.

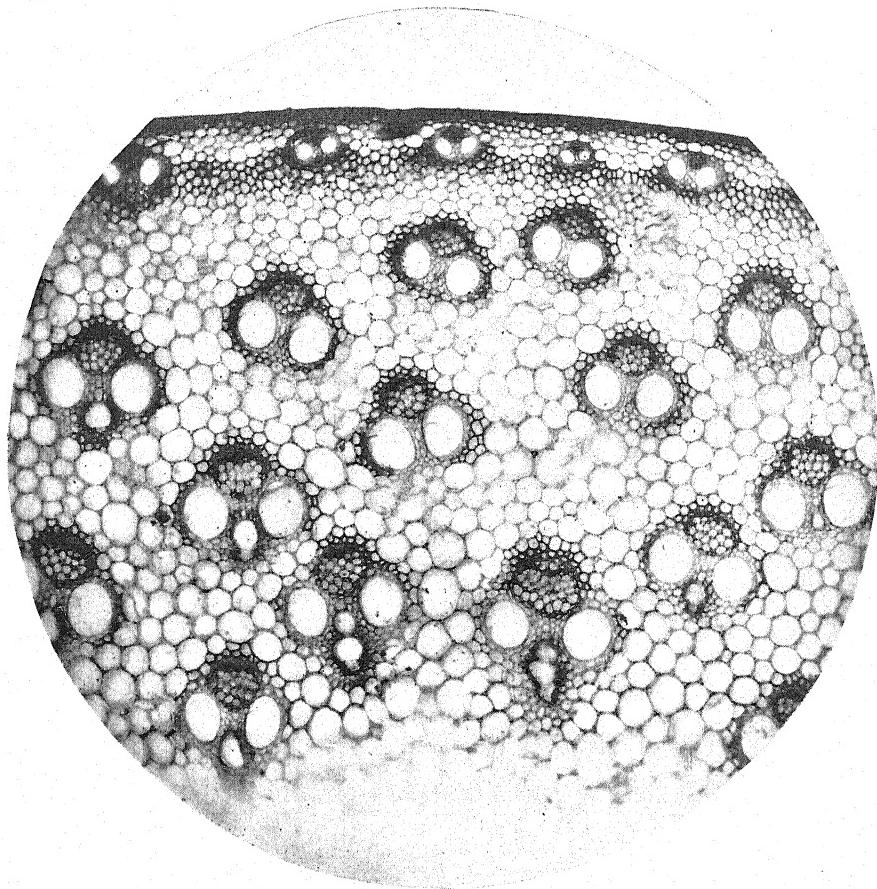


PLATE I. STEM OF CANE, TRANSVERSE SECTION
MAGNIFICATION 85x

Medium-power views of botanical subjects, such as sections of plant stems, offer a fine starting point for beginners in biological photomicrography. They are usually contrasty and more easily interpreted than animal tissues, because of the continual repetition of the structural elements. With respect to the spectacular aspect, often an asset in the first pictures taken, endogenous stems, such as that shown here, are superior to the exogenous.

Sharp delineation of the structures — cell walls, etc. — will be one indication of a successful micrograph.

Exposure Data

Objective — 16 mm. (10x) apochromat

Eyepiece — Homal II

Condenser — 1.4 N.A. aplanat

Section stained light red

Illumination — 500-watt lamp

Plate — Wratten M

Filter — B (green)

Exposure — 3½ seconds

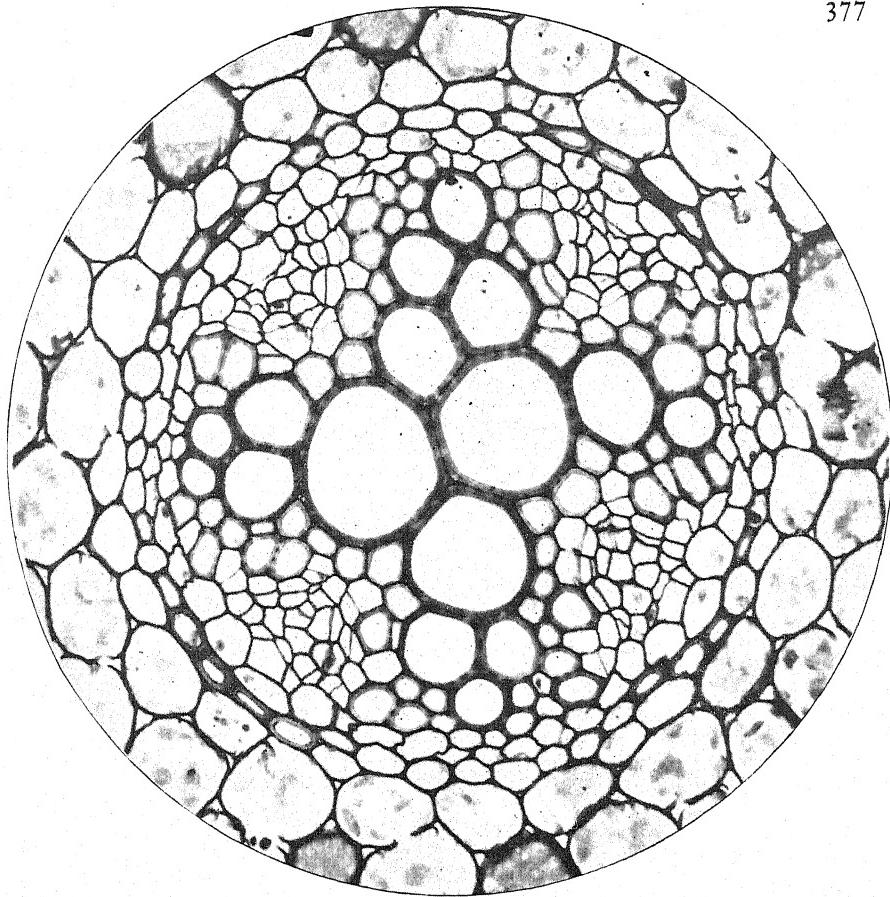


PLATE II. VASCULAR CYLINDER IN ROOT OF RANUNCULUS,
TRANSVERSE SECTION
MAGNIFICATION 300 \times

When success has been achieved with low-power pictures, an increase in magnification is the next logical step. A single fibrovascular bundle will serve as a suitable subject. It should be enlarged to the point where each type of cell can be differentiated easily from the others.

This micrograph, showing the central vascular cylinder in the root of the common buttercup, gives a good idea of how a satisfactory view at a medium high power should appear. The contrast between the clear background and the various constituents of the section should be sufficient to eliminate a muddy or foggy appearance, yet there should be ample difference in the cells so they do not all appear a uniform black.

Exposure Data

Objective - 16 mm. (10 \times) apochromat

Eyepiece - Homal I

Condenser - 1.4 N.A. aplanat

Section stained with hematoxylin and safranin

Illumination - 500-watt lamp

Plate - Wratten M

Filter - G (orange)

Exposure - 25 seconds

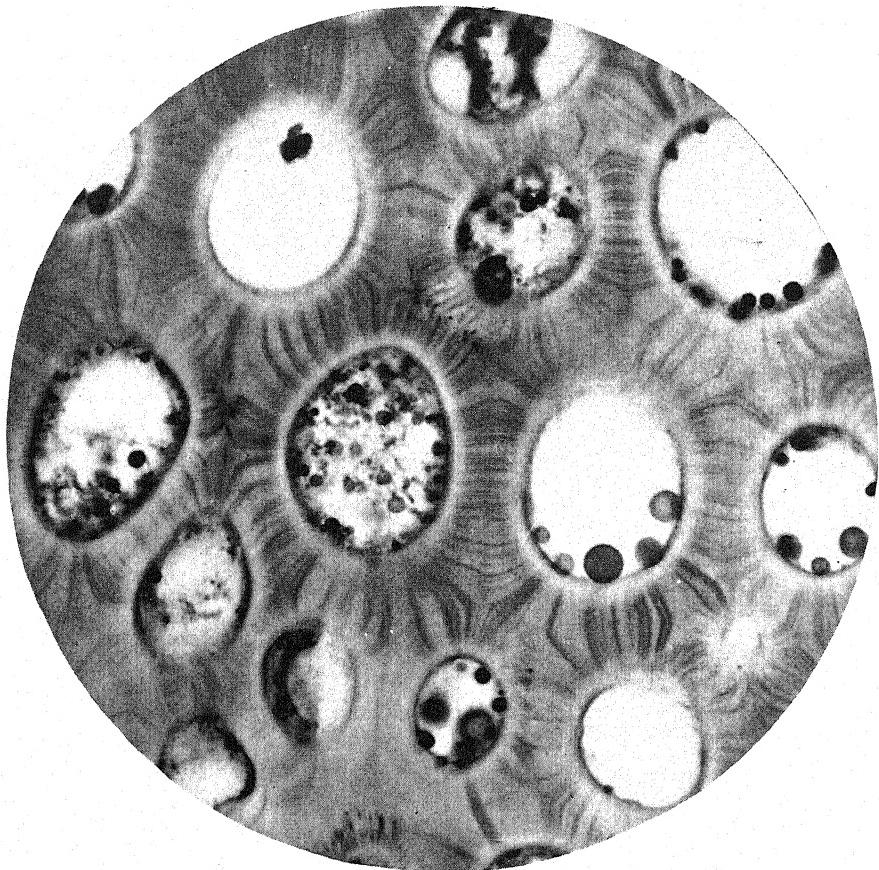


PLATE III. PROTOPLASMIC THREADS IN SEED OF DIOSPYROS
MAGNIFICATION 750 \times

The photography of a botanical section such as shown in this micrograph represents a further advance in the technical problems involved. Not only is the magnification considerably higher — made necessary by the fineness of the protoplasmic threads between the cells, which constitute the particular item of interest in the subject — but careful attention must be given to securing a semi-optical section.

The seed of *Diospyros*, the Japanese persimmon, is composed of sclerotic cells (i.e., like a date seed), but passing through the bonelike structure of the cell walls are numerous capillaries serving to connect the protoplasm of the adjacent cells and provide communication between them. Special staining technique is necessary to make these threads visible. They are analogous to the canaliculi in bone (Plate 10).

Exposure Data

Objective — 8 mm. (20 \times) apochromat

Eyepiece — Homal I

Condenser — 1.4 N.A. aplanat

Section stained with iron hematoxylin

Illumination — 500-watt lamp

Plate — Wratten M

Filter — G (orange)

Exposure — 15 seconds

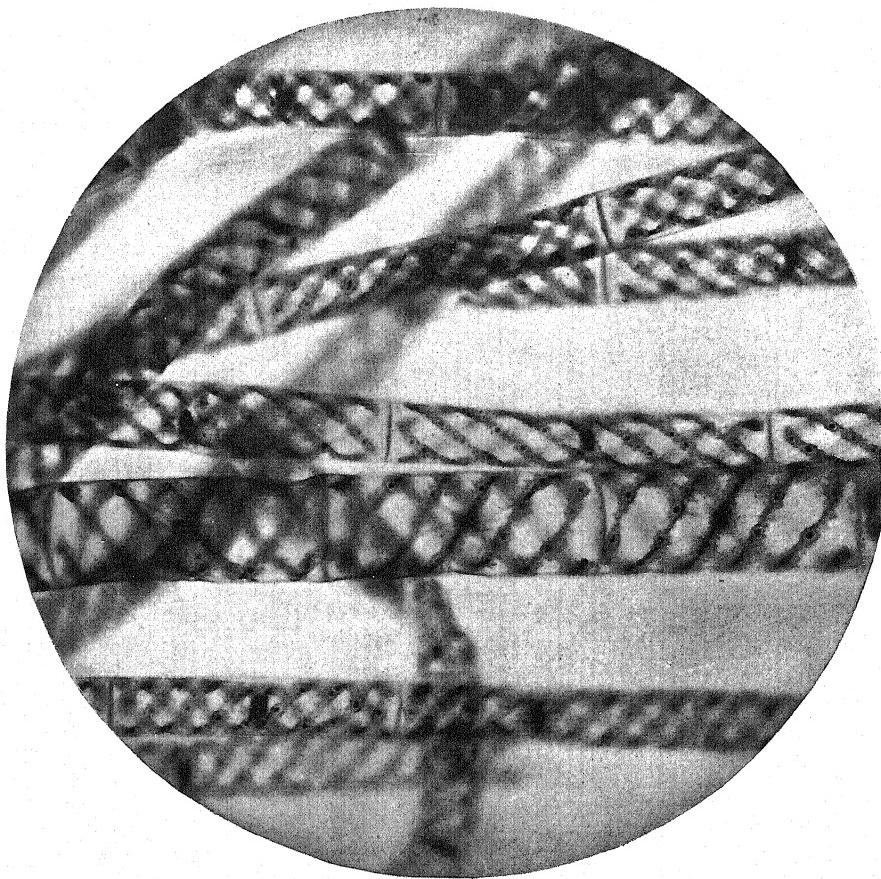


PLATE IV. SPIROGYRA
MAGNIFICATION 230 \times

In spirogyra, the slimy "fog spittle" of ponds and ditches, the microscopist finds one of the most beautiful of objects. The cylindrical filaments with their green spiral coils, the spaced pyrenoids and the centrally placed nucleus, with its radiating protoplasmic threads, offer the photomicrographer a fine opportunity to test out his skill.

A picture of this alga, to be successful, should reveal all the structural details in such a manner that a natural appearance results.

Exposure Data

Objective 16 mm. (10 \times) apochromat
Eyepiece — Homal I
Condenser — 1.4 N.A. aplanat
Specimen stained with methyl violet

Illumination — 500-watt lamp
Plate — Wratten M
Filter — B (green)
Exposure — 25 seconds

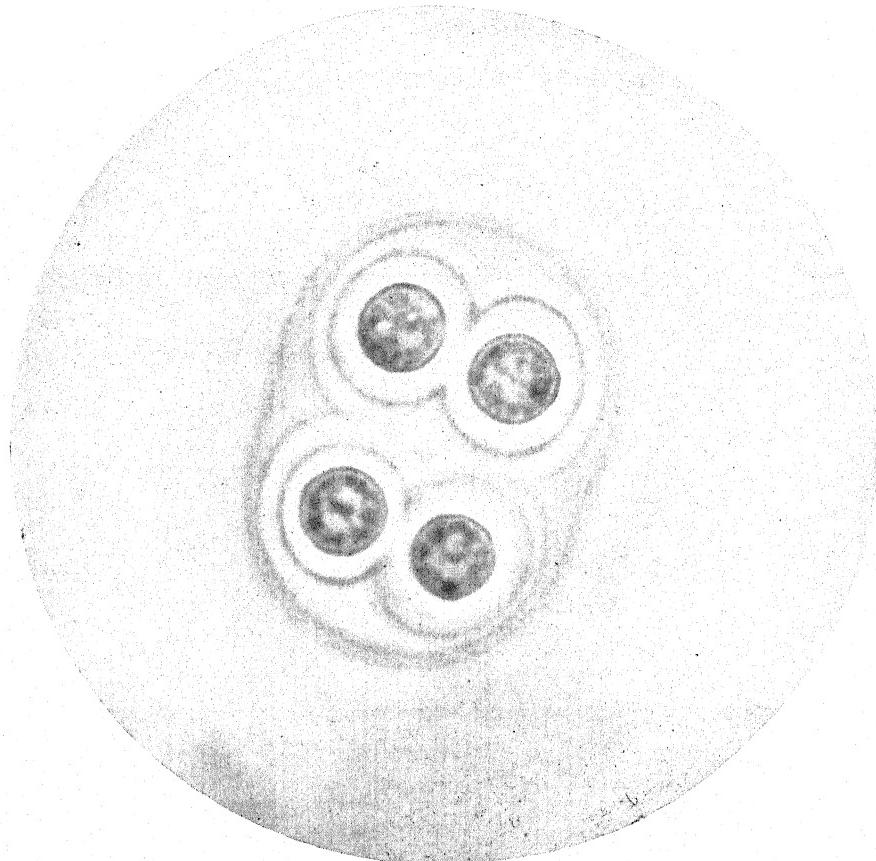


PLATE V. GLOEOCAPSA
SHOWN IN THE LIVING STATE
MAGNIFIED 2000 \times

Gloeocapsa is a minute unicellular alga, somewhat colonial in habit because of the presence of a thick gelatinous capsule surrounding the cells. This holds them together in groups of two, four, etc. The cell itself contains chlorophyll, and hence is green in color.

The specimen shown here was photographed in the living state, unstained, in water. When four individuals occur together, as is the common condition, there are three separate capsules present. A good micrograph should show these. It is easily accomplished by stopping down the cone of illumination with the substage diaphragm.

This is one type of photomicrography that requires the use of the microscope in a vertical position.

Exposure Data

Objective - Zeiss X apochromat (3mm. .85 N.A.)

Eyepiece - Homal III

Condenser - 1.4 N.A. aplanat, reduced aperture

Specimen living, mounted in fresh water

Illumination - 500-watt lamp

Plate - Wratten M

Filter - G (orange)

Exposure - 2½ minutes

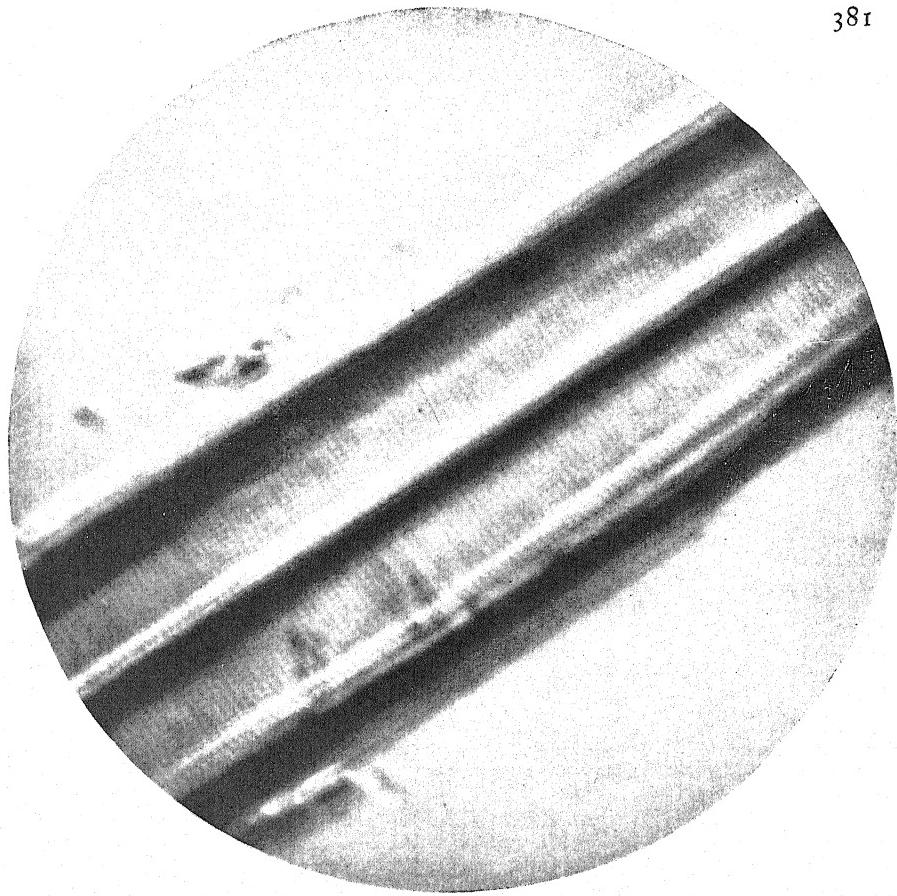


PLATE VI. AMPHIPLEURA PELLUCIDA
MAGNIFICATION 4500X

Amphipleura pellucida has long been the classic test for high-power objectives and for determining the skill of the microscopist. To be able to resolve the markings into transverse lines is a feat requiring considerable ability, while to show the dots represents the utmost in microscopy, both on the part of the lens and its possessor. At least, so thought the old-time microscopists. The fact that specimens from different localities vary greatly in the ease with which they can be resolved into dots has been generally recognized. This difference materially discounts the results achieved in any case, either in visual or photomicrographic work.

Of all the slides of this diatom in the author's collection, mounted in all sorts of media, some cannot be resolved into dots at all. The best specimen for dot resolution happens to be mounted in balsam, and is therefore hard to photograph so as to yield good contrast. The specimen shown here, at a direct magnification of 4500x, is mounted in realgar, but is only mediocre so far as dot resolution is concerned. Nevertheless the dots can be seen in the picture. The use of light of shorter wave length increases the resolution but this cannot be done with realgar mounts because realgar is a strong yellow filter and cuts off all ultra-violet light.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochro-mat

Illumination — 10 ampere arc
Plate — Hammer slow

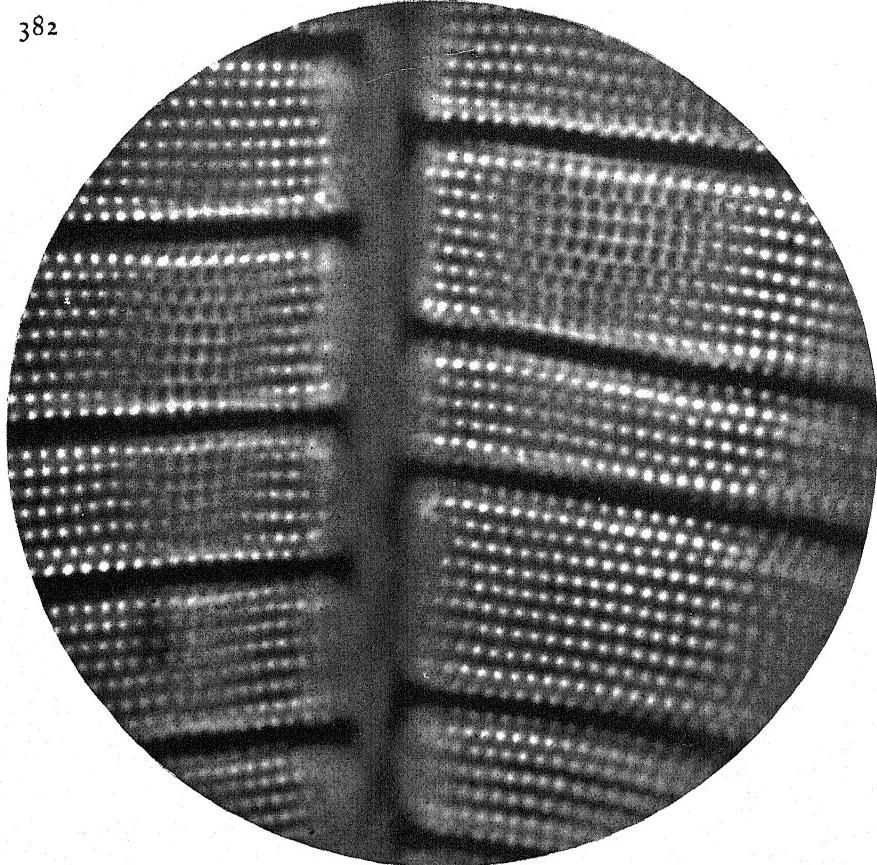


PLATE VII. DIATOM, SURIRELLA GEMMA
MAGNIFICATION 6000 \times

The photography of diatoms probably offers a greater temptation to go the limit in the matter of empty magnification, than any other field of microscopy. To obtain resolution of the markings on some of the finer-marked species is, in itself, a cause for gratification. Naturally, therefore, to reveal these markings plainly in a high-power micrograph is a further goal toward which to strive.

Surirella gemma is not a difficult diatom to resolve, for the markings run around 60,000 to the inch. Neither is it a difficult diatom to photograph, although the surface is very uneven. But to obtain a direct magnification of 6000 \times , without an objectionable amount of fuzziness, requires the best quality of objective and a good photomicrographic outfit. This micrograph is included here to illustrate what can be accomplished, should one's inclination lie toward the realm of super-power magnifications.

Exposure Data

Objective - 2 mm. 1.4 N.A. apochro-
mat

Eyepiece - Zeiss #12 compensating

Condenser - Watson Holos, oiled, ax-
ial light

Illumination - 5 ampere arc

Plate - Wratten M

Filter - C (blue-violet)

Exposure - 1 minute

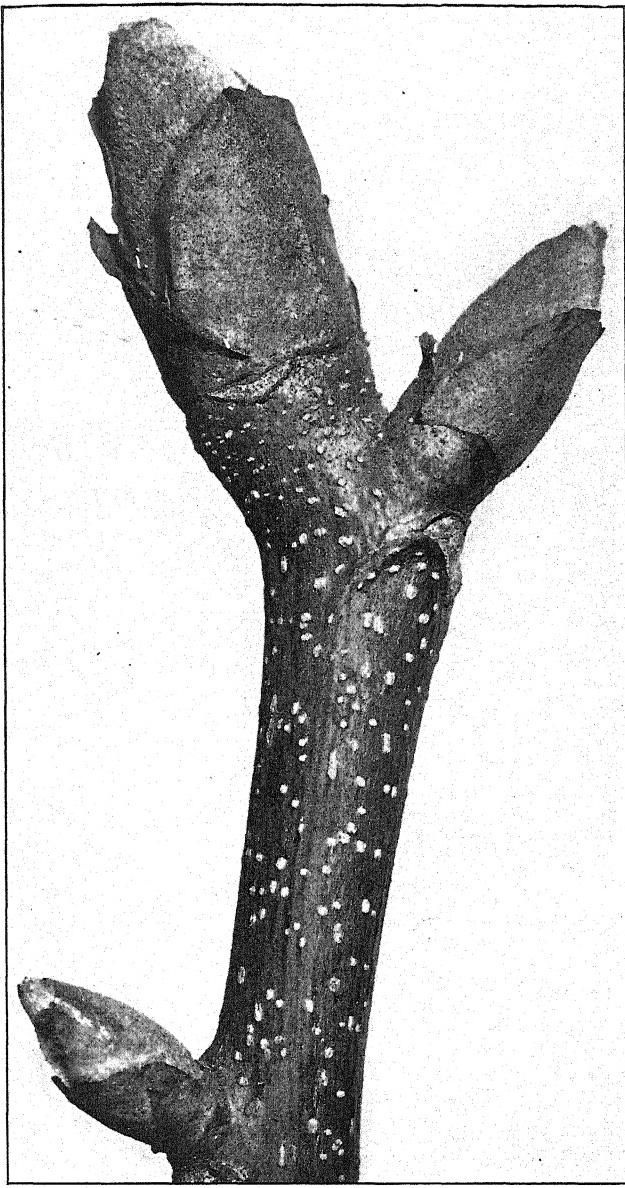


PLATE VIII. LEAF BUD OF HICKORY

MAGNIFICATION $4x$

An opaque object such as this offers the photomicrographer an opportunity to meet unusual conditions in low-power work. In the first place, the stem is cylindrical and of considerable diameter, so that the depth of focus required is excessive. The next problem is to illuminate the stem so that no part will be in deep shadow, and at the same time the terminal buds must show suitable relief. The background must be such that it will not detract from the object. Diffuse lighting, a long-focus objective, working at a small aperture, and a correct exposure, provide the answer. The background is a large white cardboard, placed sufficiently far away that no shadows can fall upon it, though it must be amply illuminated by the light source.

Exposure Data

Objective — 100 mm. Planar at F:16
Illumination — one 400-watt lamp andPlate — Wratten M
Filter — none

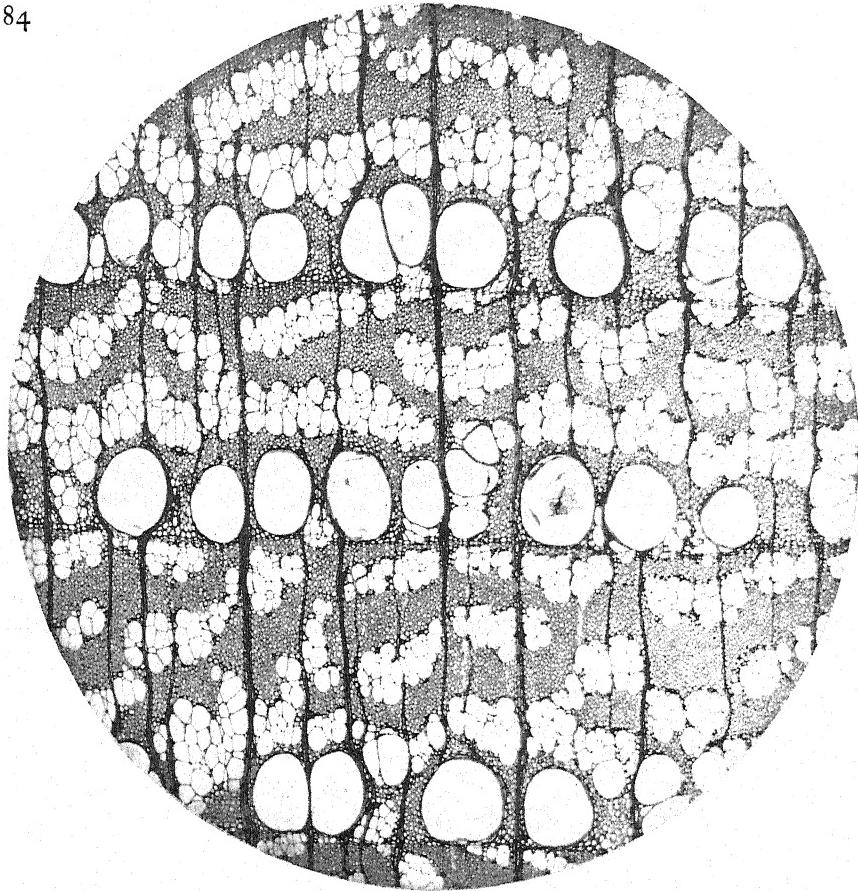


PLATE IX. SLIPPERY ELM WOOD (*ULNA FULVA*), TRANSVERSE SECTION
MAGNIFIED 40 \times

For identification purposes the transverse section of wood is the preferable one to photograph. It requires three sections, however, to reveal the complete structure, a radial and tangential as well as transverse.

Low magnification, such as this, will depict the annual rings, structural characteristics, etc., better than a higher power. For study of the individual cells it is necessary to use a higher magnification.

The photomicrography of wood sections usually is not different from that employed for other botanical sections. Some sections may be unstained and the necessary contrast can be secured only by the use of non-color-sensitized plate and a reduction in the illumination cone. There is frequently a yellow tint in the lignin which photographs best on plates sensitive to blue rays only. At other times woods are deeply colored brown or red and require a red-sensitive and possibly a red filter.

Exposure Data

Objective — 20 mm. Planar

Illumination — 500-watt lamp

Condenser — 2 cm. Spectacle lens, using Köhler low-power method

Plate — Wratten M

Filter — B (green)

Exposure — 2 seconds

Section stained with safranin

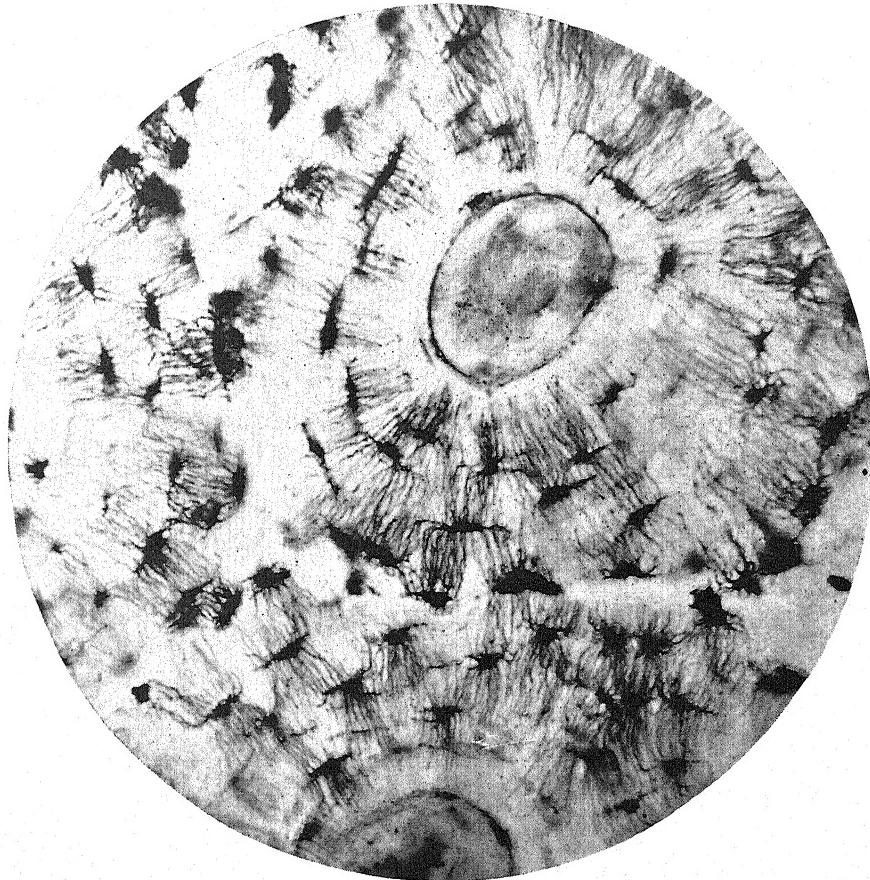


PLATE X. TRANSVERSE SECTION OF HUMAN TIBIA
MAGNIFICATION 300 \times

The most interesting feature in bone structure is the presence of the fine *canalliculi* which radiate out into the bone from the minute *lacunae*. These are the channels by means of which the fluid communication is established between the various cells. A good microscopical preparation is necessary in order to reveal them properly. Then the photomicrograph, to be of any value, must be of sufficient magnification to separate the individual processes.

For a beautiful analogy to bone structure, in the vegetable kingdom, see Plate III, where the protoplasmic threads in a hard seed are not only quite similar in structure, but perform the same function.

Exposure Data

Objective — 16 mm. (10 \times) apochromat

Eyepiece — Homal I

Condenser — 1.4 N.A. aplanat

Illumination — 500-watt lamp

Section ground, silver impregnated and counterstained red

Plate — Wratten M

Filter — B (green)

Exposure — 40 seconds

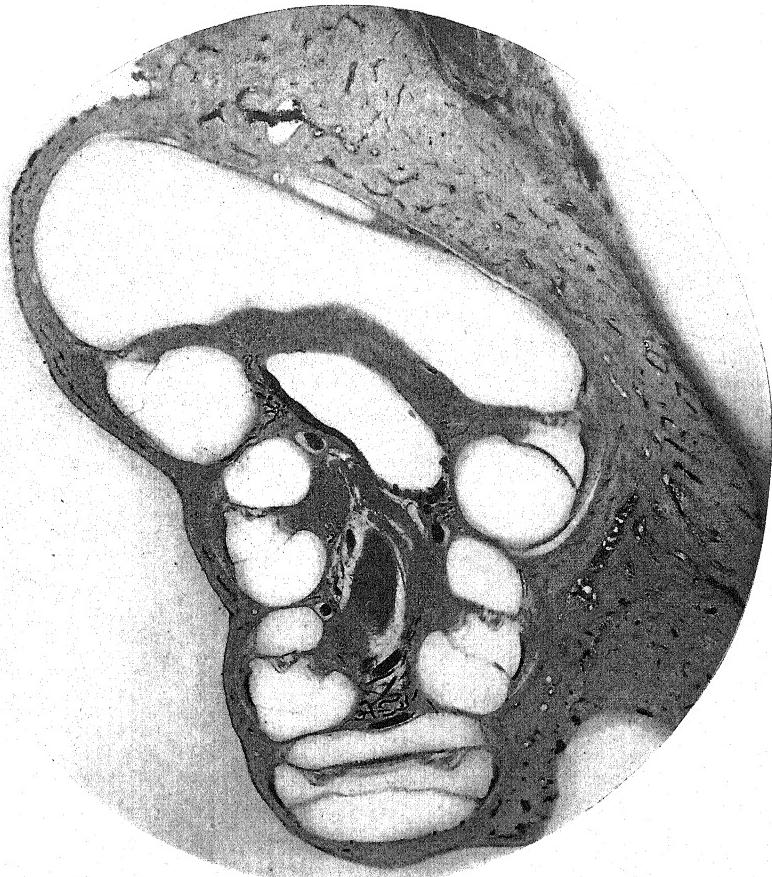


PLATE XI. COCHLEA OF GUINEA PIG, SHOWN IN LONGITUDINAL SECTION
MAGNIFICATION $25x$

In intricacy of design the auditory organ is a close second to the eye. That part of the ear where sound vibrations are transformed to nerve sensations resembles a minute convoluted sea-shell. This micrograph gives a good idea of the general form of this intricate apparatus, the cochlea, as it appears in a median longitudinal section.

The magnification is not sufficient to reveal the complexities of design but it is that best suited to convey an idea of the structure as a whole. This type of photomicrograph, covering a unit structure in its entirety, fills an important niche in biological photomicrography.

Exposure Data

Objective — 20 mm. Planar at F:6.3

Illumination — 500-watt lamp

Condenser — Zeiss #17 spectacle lens

Section stained with hematoxylin and eosin

Filter — B (green)

Plate — Wratten M

Exposure — 4 seconds

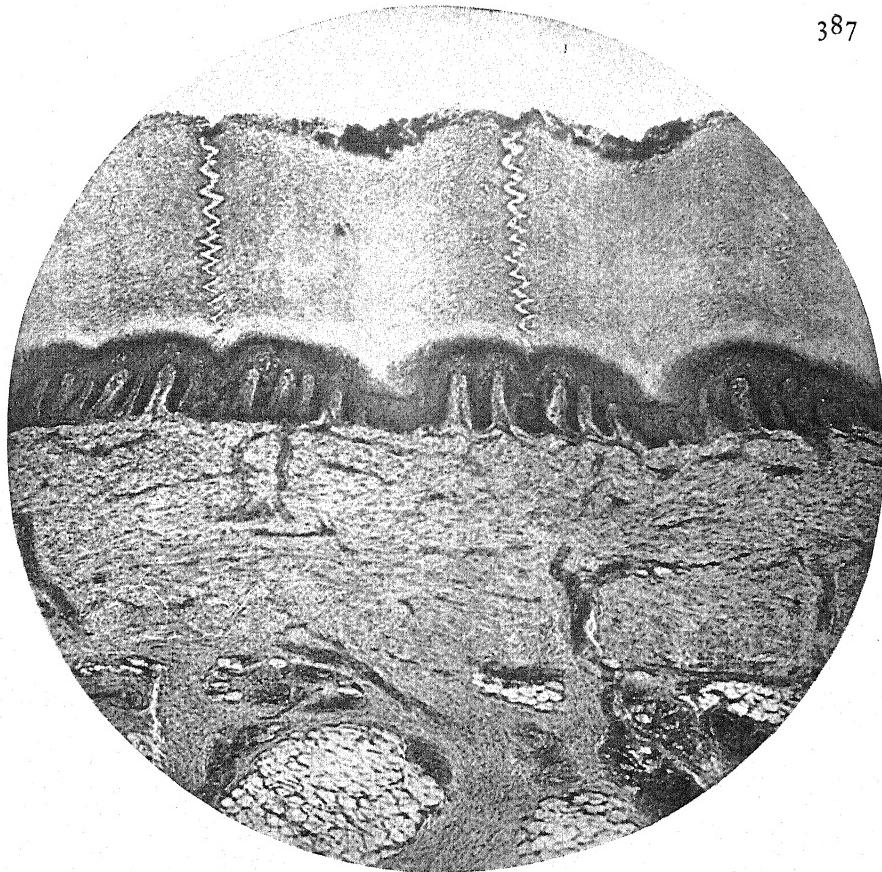


PLATE XII. SPIRAL SWEAT DUCTS IN SKIN
MAGNIFICATION 40 \times

This is a type of subject requiring special technique to bring out the presence of the spiral sweat ducts which pass through the thick outer portion of the skin on the palm of the hand and sole of the foot. The possibility of showing them as in this micrograph arises from the fact that the walls of the ducts possess a slightly higher refractive index than the surrounding tissue. On account of their spiral nature they have an appreciable depth, so that a low-power lens must be used to get them in focus. Then, by further reducing the aperture of the illuminating cone, the difference in the refraction brings them prominently into view.

The section is purposely cut thick, to include the complete spiral and hence, with reduced aperture, the cells of the rest of the tissues cannot be well shown at the same time. The depressions on the surface of the skin, constituting the "finger-print" structure, are evident. They are partly filled with dirt.

Exposure Data

Objective - 20 mm. Planar

Condenser - 2 cm. spectacle lens

Köhler Illumination with reduced aperture

Section stained carmine

Plate - Wratten Panchromatic

Filter - G (orange)

Exposure - 1½ seconds

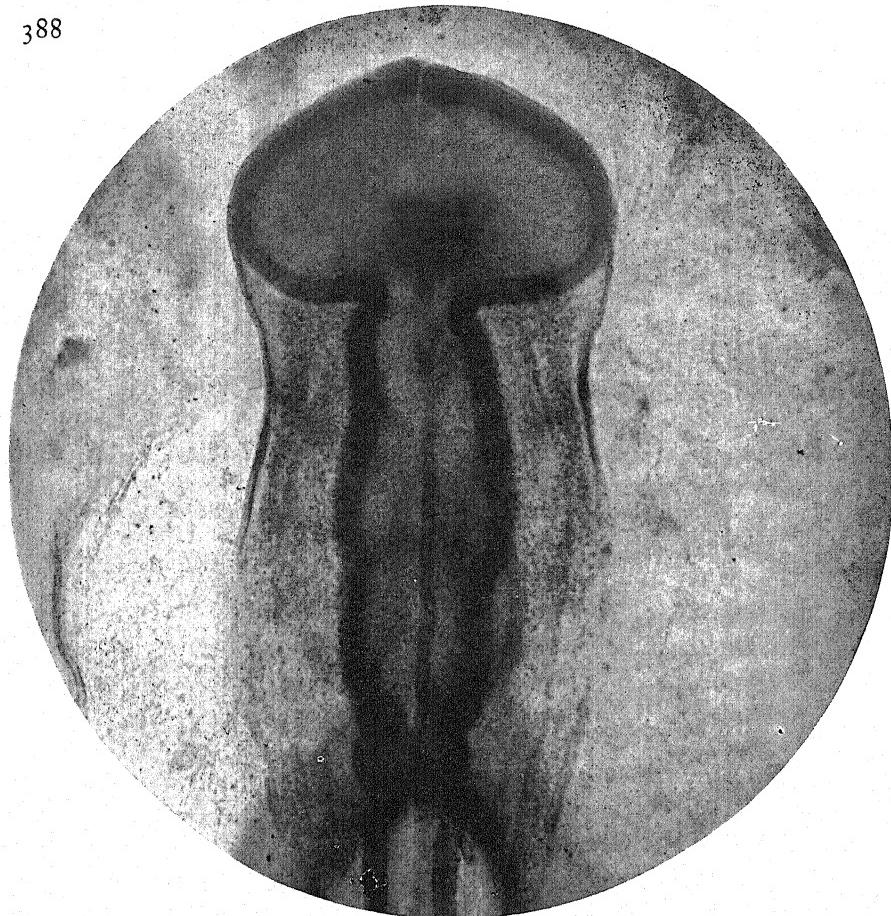


PLATE XIII. 33-HOUR CHICK EMBRYO, WHOLE MOUNT
MAGNIFICATION 75 \times

In the field of embryology the photomicrographer can find abundant material suitable to his needs. A series of stages in the development of a chick embryo, from the primitive streak to the fully formed body, provides pictures of the highest educational value for teaching purposes. In the stage shown in this micrograph the early development of the fore-, middle-, and hind-brain; the thickness of the neural tissue; the still open anterior cleft between the neural folds; the formation of the primary optic lobes; and the early condition of the ectoderm and mesoderm, can all be demonstrated.

The technique involved in photographing an entire series of stages is quite varied and extensive, the complexity of the subject increasing all the way from the primitive streak to the age where whole mounts can no longer be considered in a microscopical classification.

Exposure Data

Objective — 6 \times apochromat

Eyepiece — Homal II

Condenser — 1.4 N.A. aplanat, with
top removed

Stain, faint hematoxylin

Illumination — 500-watt lamp

Plate — Wratten M

Filter — B (green) plus G (orange)

Exposure — 6 seconds

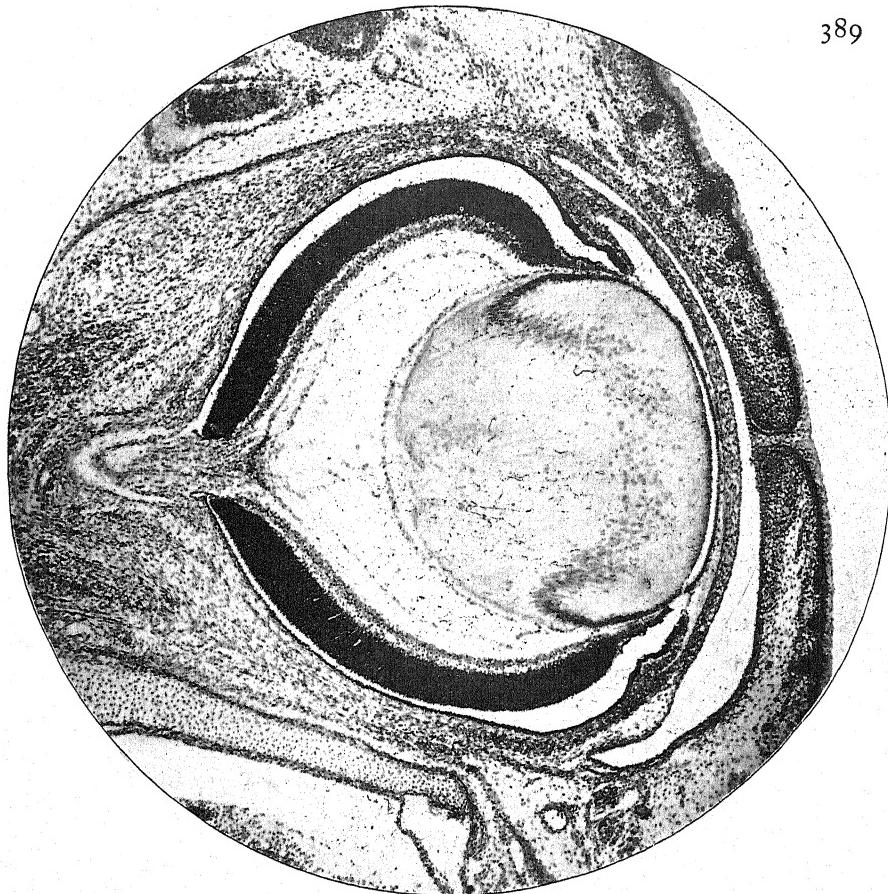


PLATE XIV. EYE OF 30 MM. RAT EMBRYO, MEDIAN SECTION
MAGNIFICATION 50 \times

In the entire realm of life, the eye is paramount in its function, marvels of structure, and multiplicity of unusual histological elements. Satisfactory photomicrographs of the eye depend in part upon the quality of the microscopical preparation.

The picture shown here owes a large measure of its educational value to the perfection of the section. The photomicrographic technique itself is not out of the ordinary and the subject as a whole is illustrative of average histological material. Its chief contribution to this series lies in emphasizing the advantage of good microscopical preparations, when they are to be photographed. Poor sections cannot be expected to result in good pictures. The stain of this section was light, but compensated for by the use of a contrast filter.

Exposure Data

Objective — 5 \times apochromat
Eyepiece — Homal II
Condenser — 1.4 N.A. aplanat, with
top lens removed
Section stained with carmine

Illumination — 500-watt lamp
Plate — Wratten M
Filter — B (green)
Exposure — 1½ seconds

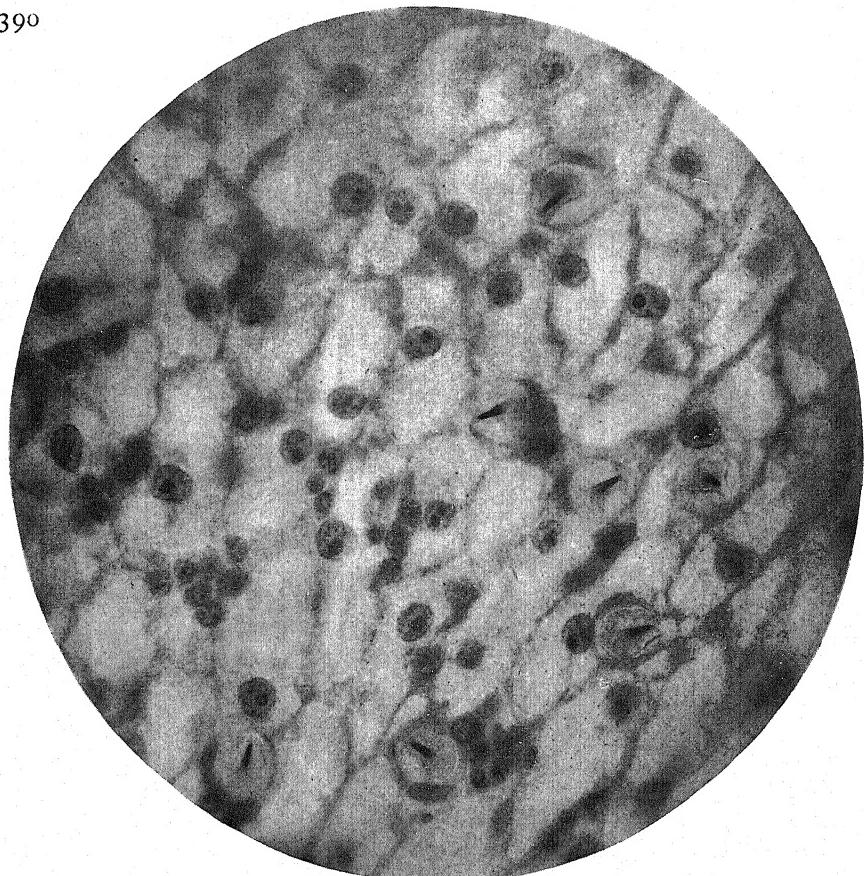


PLATE XV. NERVE PLEXUS IN HYDRA, SHOWN IN OPTICAL SECTION THROUGH ENTIRE ANIMAL
MAGNIFICATION 650 \times

About the most primitive neural tissue known is the nerve plexus (or network of nerve cells) occurring between the body cells of the Hydra. There are several different types of cells present in the two-layered body sac, each with its own individual function. The nerve plexus serves to connect all these together so that the Hydra body as a whole functions as an individual organism.

The photomicrographic problem in this case, with the help of staining methods which differentiate the nerve plexus, is to penetrate the body layers of an entire animal (four layers deep, counting the two sides) and isolate the plexus so that it will appear as such in the micrograph, without being complicated by an excess of other cells. On the whole, this is an extremely difficult picture to produce.

Exposure Data

Objective — Zeiss X apochromat (3 mm., .85 N.A.)

Eyepiece — Homal II

Condenser — 1.4 aplanat at .85 N.A.

Object stained pale blue and red

Illumination — 500-watt lamp

Plate — Wratten M

Filter — G (orange)

Exposure — 5 seconds

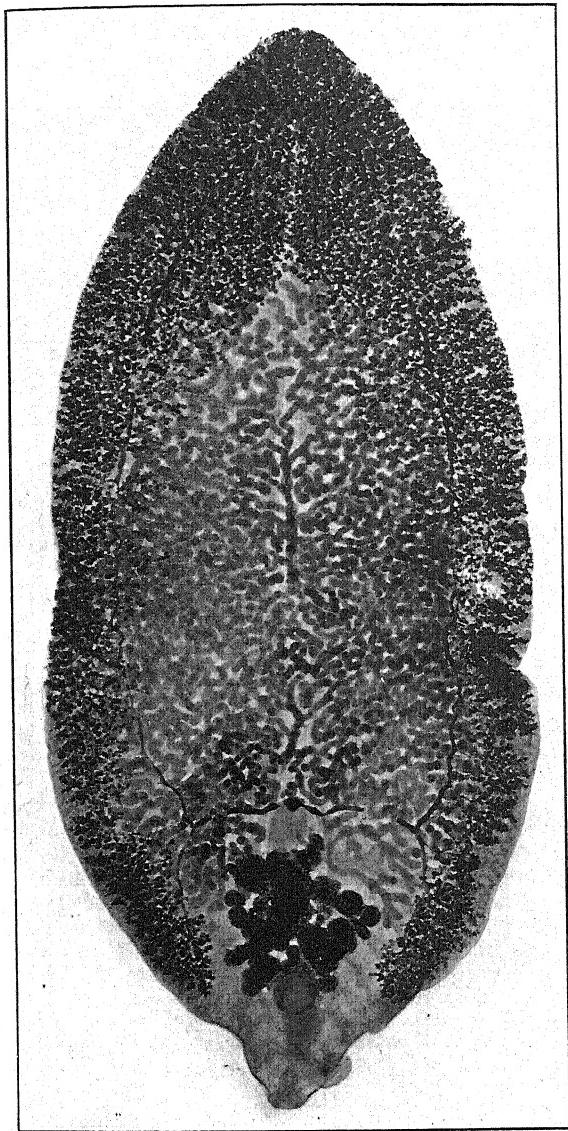


PLATE XVI. LIVER FLUKE, DISTOMUM HEPATICUM

MAGNIFICATION 5X

This is the type of object requiring a very low-power, long-focus lens, and an illuminating system which will satisfactorily cover the entire area. Many microscopes do not possess a sufficiently large opening in the stage to accommodate the object. The lens must then be mounted on the lens board and the microscope removed entirely. If the photomicrographic outfit is not provided with means for supporting and illuminating a slide without the use of the microscope, these must be improvised.

Exposure Data

Illumination — 500-watt lamp using Köhler method
Exposure — $\frac{1}{2}$ second Filter — A (red)
Plate — Wratten M

Objective — 100 mm. Planar
Condenser — Zeiss #12 spectacle lens
Object stained deep carmine

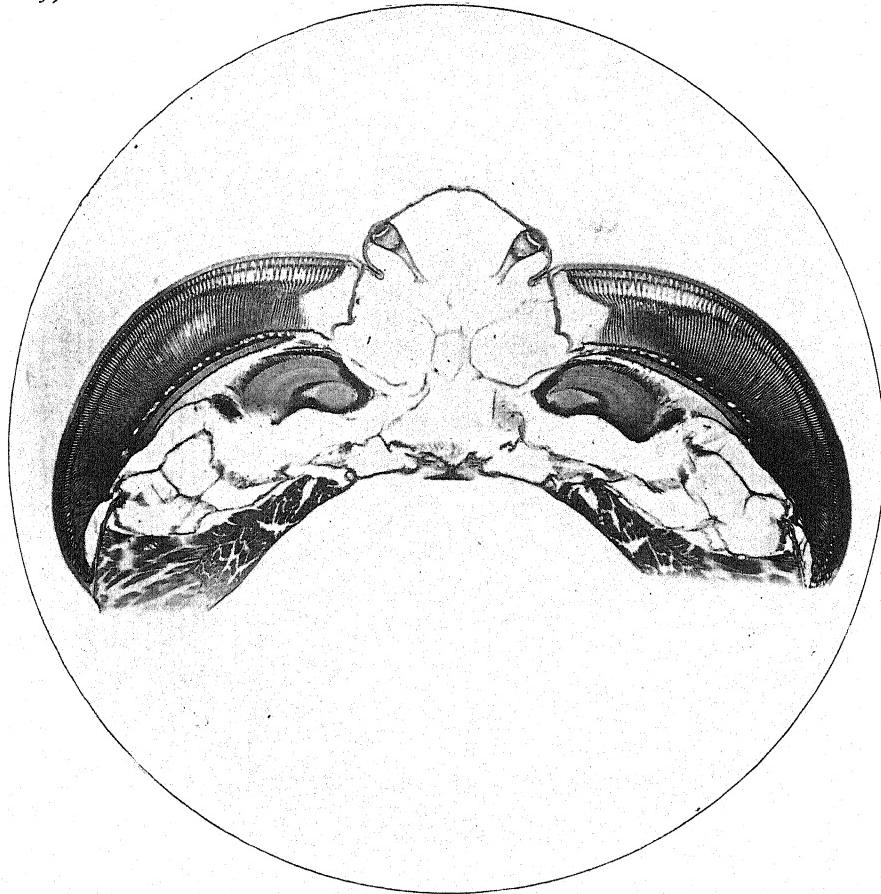


PLATE XVII. OCCELLI AND COMPOUND EYES OF DRAGONFLY
MAGNIFICATION 15 \times

Fortunate indeed is the microscopist when he obtains an occasional section which is ideally diagrammatic. The section through the head of a dragonfly shown here falls into this class. It is not only median through the ommatidia of the compound eyes but also median through the two horizontally placed ocelli.

Sections of this size require a low-power photographic lens to cover them. In this particular section the staining was quite deep, resulting in a contrasty negative.

Exposure Data

Objective — 50 mm. Planar
5 cm. condenser using Köhler method
Illumination — 500-watt lamp
Section stained with hematoxylin

Filter — E (light red) plus G (orange)
Plate — Wratten M
Exposure — 1½ seconds

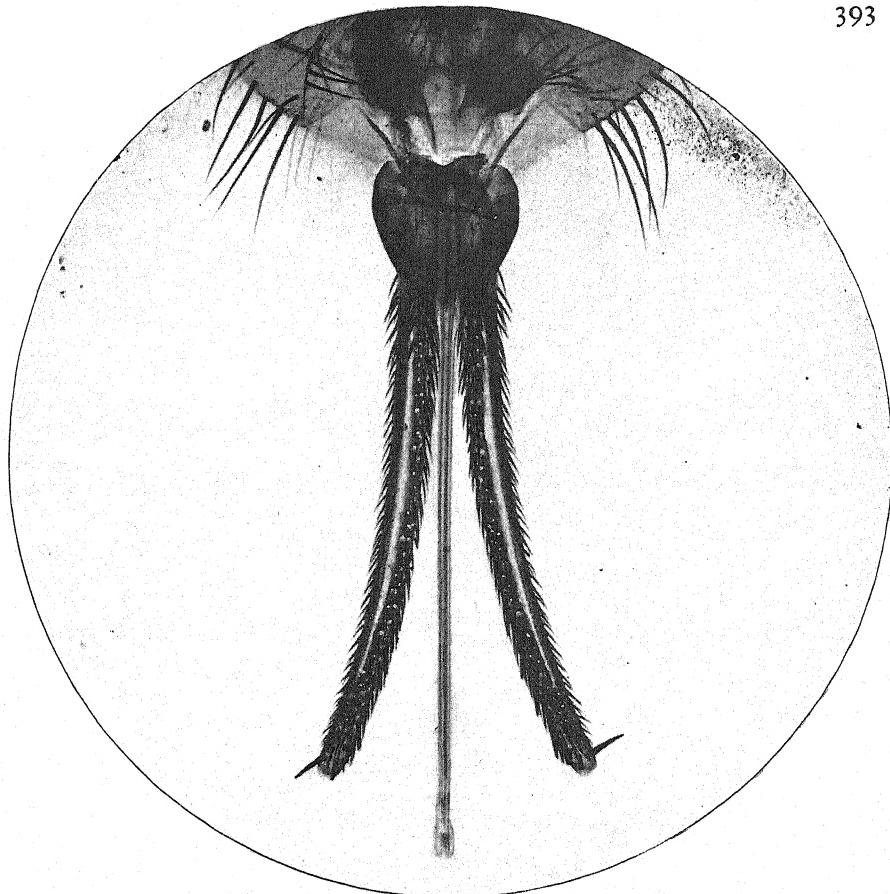


PLATE XVIII. MOUTH PARTS OF TSETSE FLY
MAGNIFICATION 30 \times

In this object we encounter one of the conditions so prevalent in the photographing of insect preparations — the great difference in the intensity of color in various portions of the chitin. The specimen shown here was mounted by the pressure method, which involves a preliminary soaking in hydroxide to soften and remove internal tissues. This results in the head (a portion of which is seen in the picture) showing beautifully clear, but the hairs and other mouth parts are still a very dark brown.

As in this case, however, we are particularly interested in the swordlike piercing beak, everything else must be subjugated to this part in taking the picture. In other words, the dark portions must be allowed to show black. Should an attempt be made to lighten them (which can be done), the beak would become practically invisible.

Exposure Data

Objective — 20 Planar, slightly stopped
Illumination — 500-watt lamp
Condenser — Zeiss #17 spectacle lens

Filter — G (orange)
Plate — Wratten M
Exposure — 1 second

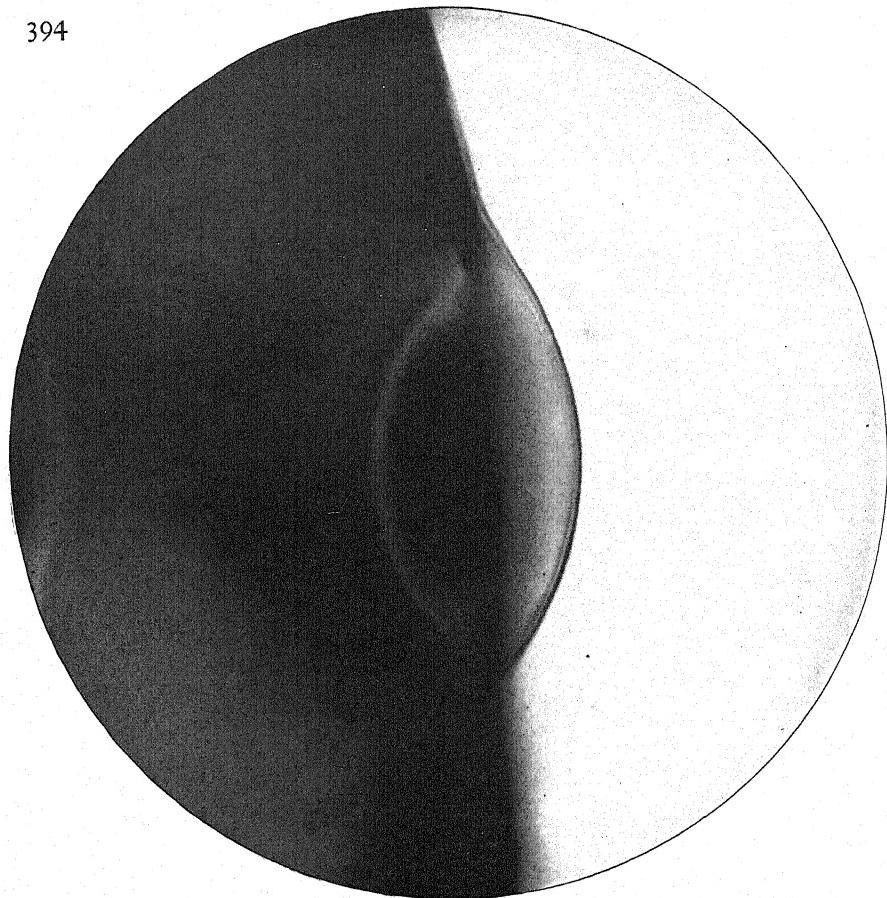


PLATE XIX. THE EYE LENS OF A BODY LOUSE, SHOWN IN OPTICAL SECTION
MAGNIFICATION 1000X

Though most insects possess compound eyes (as shown in section in Plate XVII), the "cootie," as it is familiarly called, has individual eyes, one on each side of the head. In a mounted specimen the lens stands vertically, and so provides an opportunity for observing the surface contours. To photograph it, however, is quite a problem. It requires an optical section and considerable magnification. The keratin of the exoskeleton is pale yellow in color, so that, contrary to the usual condition when photographing insects, increased contrast, instead of reduced, must be secured. The lens itself is transparent.

The micrograph is interesting in the information it yields as to the difference in curvature of the outer and inner lens surfaces. It also reveals the presence of a thin cuticle over the lens and exoskeleton, and that the latter extends slightly within the lens, possibly serving as a diaphragm to cut out the marginal rays.

Exposure Data

Objective — 8 mm. (20x) apochromat	Eyepiece — Homal I
Condenser — 1.4 aplanat	Illumination — 500-watt lamp
Plate — Hammer Slow	Filter — Schott H, light (light blue)
Magnification obtained through use of long bellows	Exposure — 75 seconds

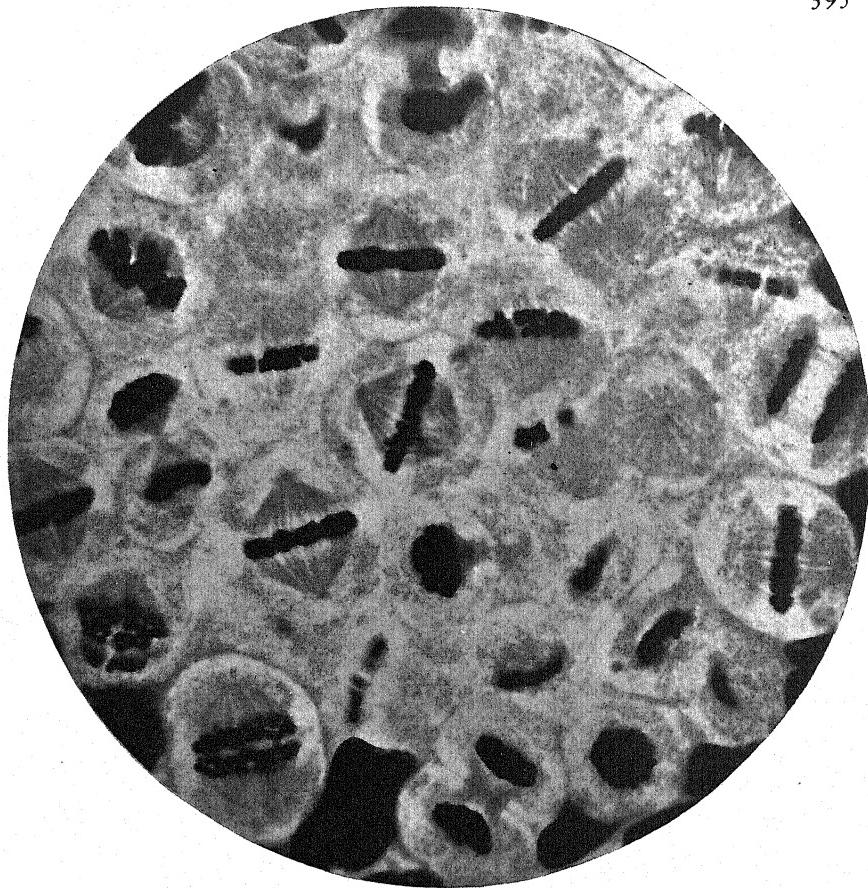


PLATE XX. MITOSIS IN SPERMARY OF POTOMOBius
MAGNIFICATION 700 \times

A general view photomicrograph should reveal as much information regarding the subject depicted as can be crowded into it. In one showing cell division, as instanced here, careful examination of an entire section will often disclose some particular area where an abundance of stages is present.

The chromosomes are very numerous in *Potomobius* and are nearly spherical, and hence cannot be separated in an equatorial view. On the other hand, the manner of separation of the two sets of chromosomes, and their reforming to produce the daughter cells, is easily seen. This micrograph is especially interesting for its depicting of metaphase, anaphase, and telophase stages.

Exposure Data

Objective — 8 mm. (20 \times) apochromat

Eyepiece — Homal I

Condenser — 1.4 aplanat

Section stained with iron hematoxylin

Illumination — 500-watt lamp

Plate — Wratten M

Filter — Schott 1 mm. H (light blue)

Exposure — 20 seconds

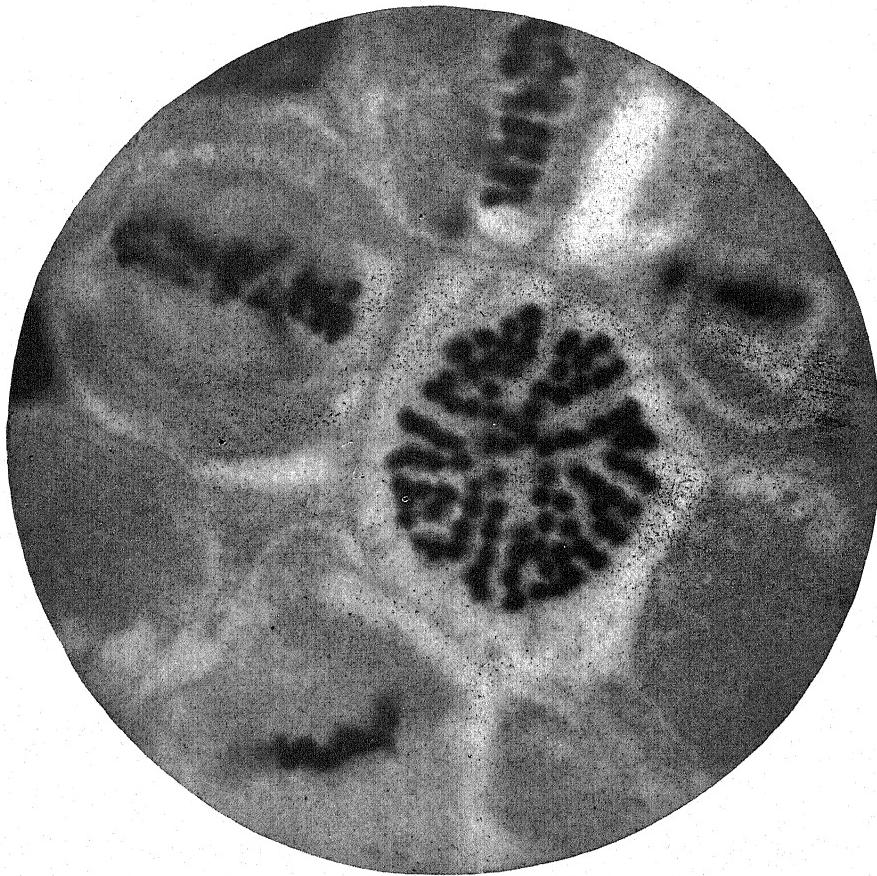


PLATE XXI. MITOSIS IN SPERMARY OF POTOMOBIUS, POLAR VIEW OF CHROMOSOMES
MAGNIFICATION 1600 \times

For a study of the individual chromosomes of *Potomobius* a polar view is necessary. Also it is desirable that the magnification be considerably higher than is required for a general view. In a photomicrograph of the chromosomes a compromise should be made in the matter of a resolution versus depth of focus. A magnification of 1600 \times requires fairly high aperture in the objective in order to avoid empty magnification, but, on the other hand, if a critically sharp focal plane, without appreciable depth, results, very few of the numerous chromosomes are in focus on any one plane.

The photography itself is relatively simple because the staining with iron hematoxylin gives a very dark color to the chromosomes regardless of the filter employed.

Exposure Data

Objective — Zeiss X apochromat (3 mm., .85 N.A.)

Eyepiece — Homal III

Condenser — 1.4 aplanat

Section stained with iron hematoxylin

Illumination — 500-watt lamp

Plate — Wratten M

Filter — Schott 1 mm. H (light blue)

Exposure — 30 seconds



PLATE XXII. MITOSIS IN HYACINTH ROOT TIP, LATE ANAPHASE STAGES
MAGNIFICATION 1600X

Probably the most interesting, as well as the most spectacular, stage in mitotic cell division is the late anaphase. At this time the chromosomes are nicely spaced into the daughter cell groups, yet retain their individuality. Visually it is sometimes possible to follow through the course of single chromosomes in this stage, especially if a stereoscopic binocular eyepiece is employed, but this cannot be done in a photomicrograph, for two reasons. First, it is impossible to alter the focus while taking a picture, as can be done for visual observation; and second, all chromosomes being stained dark, their images superimpose into one general black area. However, in a good micrograph, a compromise focus should suggest the actual structure.

Exposure Data

Objective — Zeiss X apochromat
(immersion, .85 N.A., 60x)
Condenser — 1.4 N.A. aplanat
Filter — E (light red)
Section stained with iron hematoxylin

Eyepiece — Homal III
Illumination — 500-watt lamp
Plate — Wratten Panchromatic
Exposure — 35 seconds

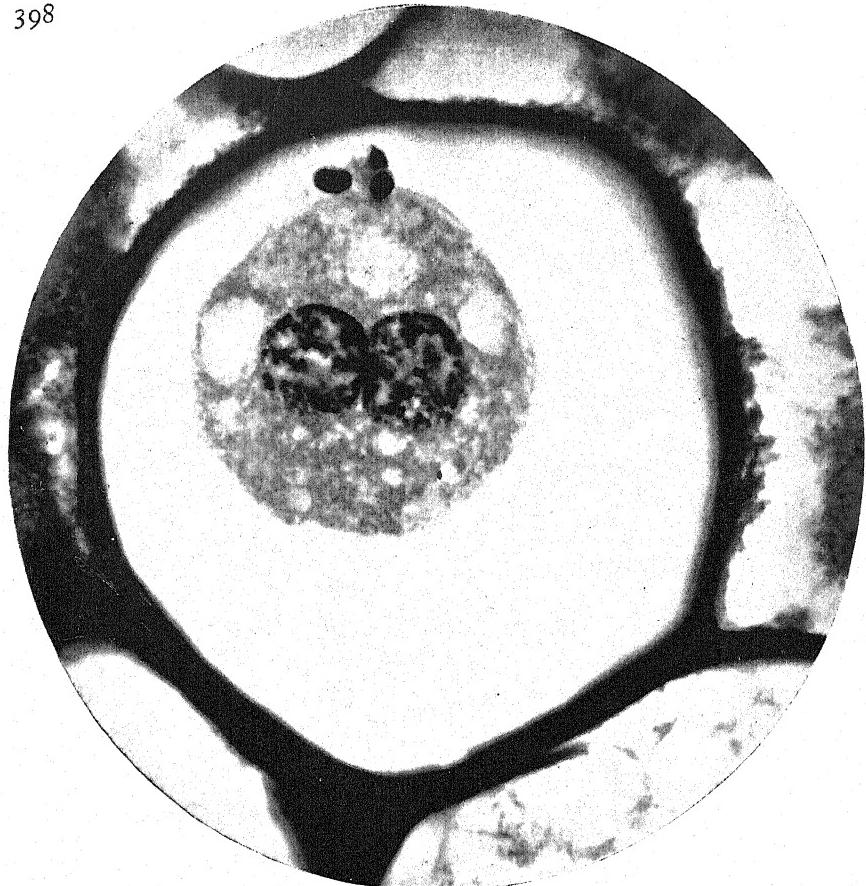


PLATE XXIII. MATURATION IN ASCARIS EGG, SHOWING THE MALE AND FEMALE PRONUCLEI

MAGNIFICATION 1200 \times

This micrograph reveals an interesting stage in the fertilization of an Ascaris egg. The male sperm has penetrated to the center of the egg cell but has not yet united with the female nucleus. The actual union does not take place until the haploid number of female chromosomes has been established.

In the picture, the three polar bodies which have resulted from previous nuclear divisions are seen at the top of the cell, where degeneration is taking place. In the center of the cell are the two nuclei which are to unite. It is impossible to state which is male and which is female. Fusion is just about to take place.

Micrographs of this type, at a fairly high magnification, are of great value for teaching purposes.

Exposure Data

Objective — Zeiss X apochromat
(immersion, .85 N.A., 60 \times)
Eyepiece — Homal III
Condenser — 1.4 N.A. aplanat
Section stained with iron hematoxylin

Illumination — 500-watt lamp
Filter — G (orange)
Plate — Wratten M
Exposure — 1 minute

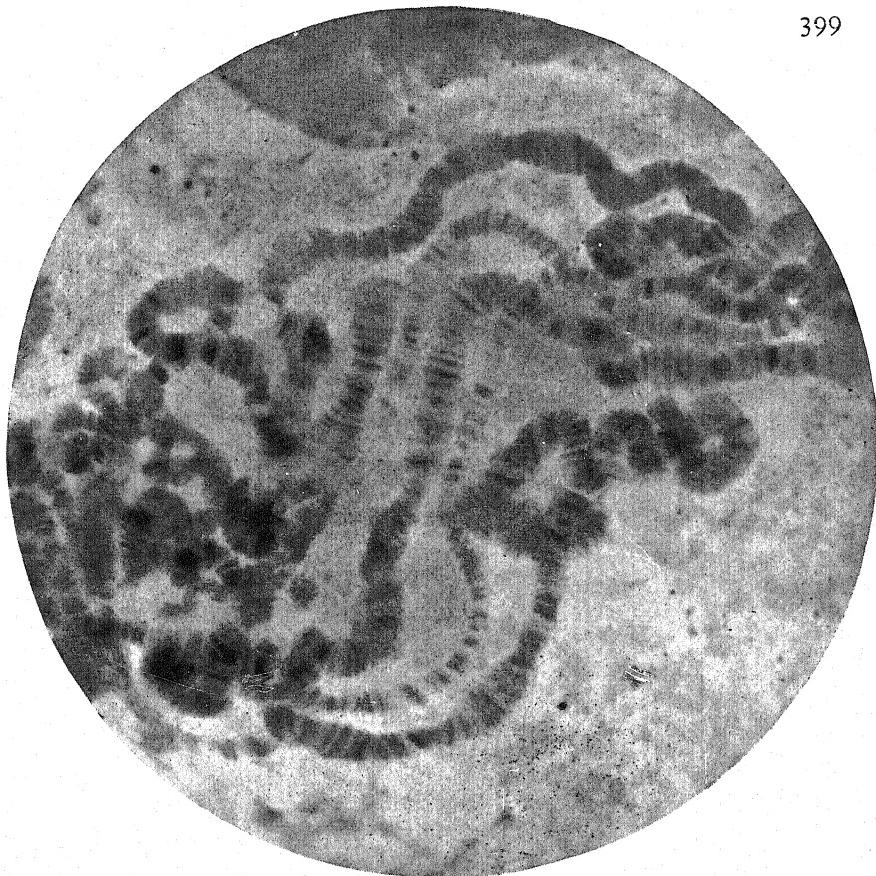


PLATE XXIV. CHROMOSOMES FROM SALIVARY GLAND OF DROSOPHILA, SHOWING THE GENES LOCI

MAGNIFICATION 1000 \times

Research work on this classical object has been the means of materially advancing our knowledge of the factors influencing heredity, inheritance, mutations, etc., during recent years. The chromosomes of the fruit fly are only four in number and are unusually large, hence easily studied. By suitable preparation methods it is possible to reveal the component parts of chromosomes from the salivary gland. These show in the picture as alternate light and dark bands transverse the chromosomes. Extensive research has identified many, at least, of the bands with distinct inherited characteristics.

The chief problem in photographing these chromosomes lies in securing sufficient contrast between the rather faintly stained bands.

Exposure Data

Objective — 4 mm. (40 \times) B. & L. immersion fluorite

Eyepiece — Homal IV

Condenser — 1.4 N.A. aplanat

Specimen stained faint red

Filter — B plus H (light)

Exposure — 18 seconds

Plate — Wratten Panchromatic

Illumination — 500-watt lamp

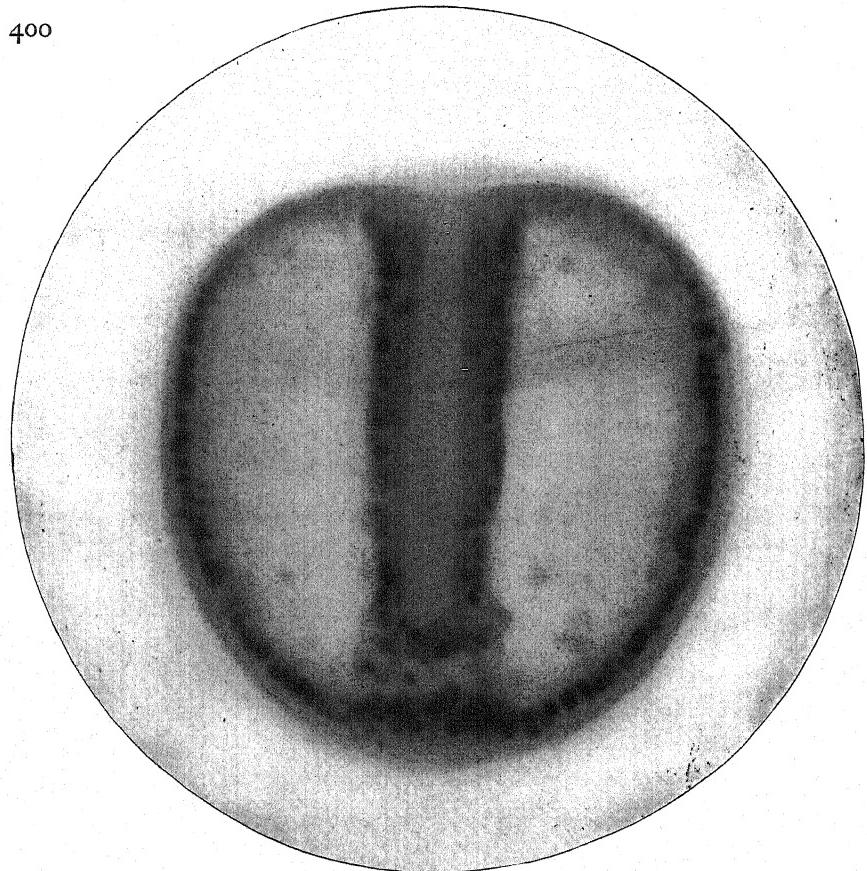


PLATE XXV. GASTRULA STAGE OF SEA URCHIN, SHOWN IN OPTICAL SECTION
MAGNIFICATION 600X

In the development of the sea urchin embryo from the single-celled egg, the first division produces two cells, and these divide into four. Increasing in geometric ratio, they become 8, 16, 32, 64, and so on. When the number of cells becomes quite numerous, they form a hollow ball, the *blastula* stage. From the more numerous cells at one place on the sphere, a hollow tube of cells starts growing internally through the center, toward the opposite side. This is known as the *gastrula* stage.

In a stained specimen, all cells of the sphere, as well as those of the inner tube, are stained alike; nevertheless, by optical sectioning, as described in the text, it is possible to photograph the inner tube and a ring of cells either above or below. There is, of course, some interference, but the picture is easily interpreted. Especially to be noted are the loose cells at the end of the tube, in the process of uniting the latter to the opposite side of the sphere, after which the hole in the tube will be opened up all the way through. The inner tube wall is plainly seen.

Exposure Data

Objective — 8 mm. (20x) apochromat
Eyepiece — #8 compensating (Zeiss)
Condenser — 1.4 aplanat at .65 N.A.
Object stained a deep pink

Plate — Wratten M
Illumination — 500-watt lamp
Filter — G (orange)
Exposure — 12 seconds

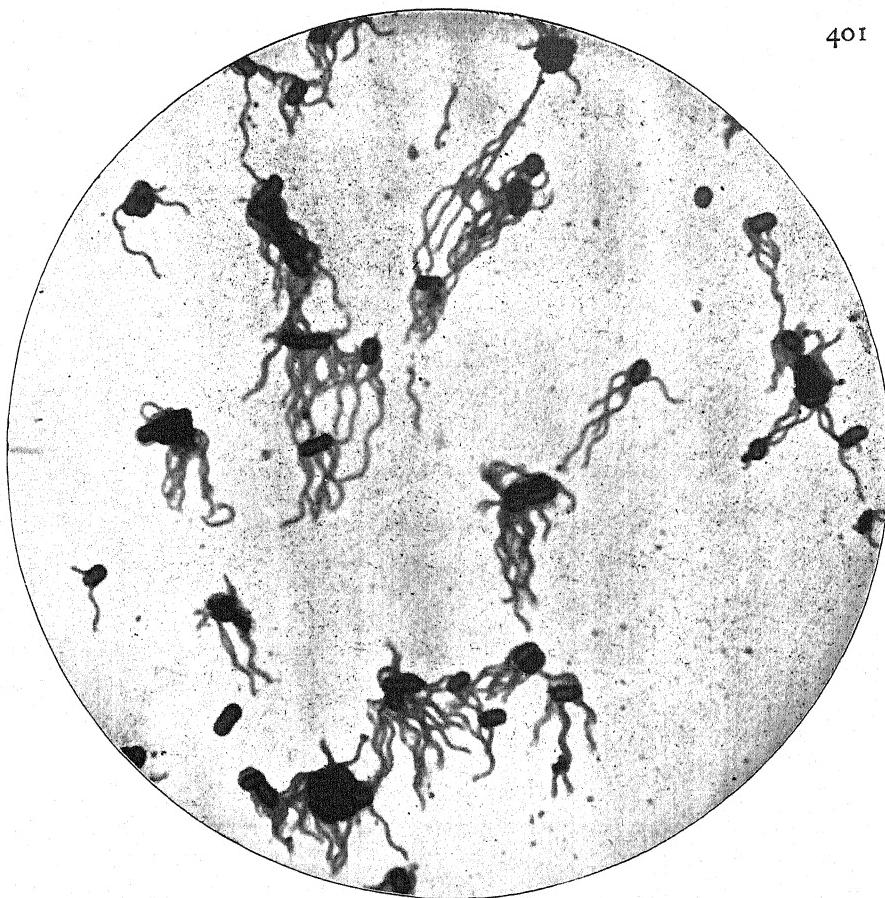


PLATE XXVI. *SALMONELLA PARATYPHI*, SHOWING THE FLAGELLA
MAGNIFICATION 2000 \times

The average pictures of bacteria one sees in various textbooks leave much to be desired. In the first place, the magnifications are usually insufficient to reveal the organisms properly; further, the photomicrographic technique is far from ideal. With the majority of species of bacteria a magnification around 2000 \times is desirable in order to convey a true conception of their form. Some need more than this, because of their extremely minute size, while on the other hand a few are so large that magnification under 1000 \times must be used.

When bacteria are shown *in situ* in tissue sections, the latter is an additional factor governing the magnification, which usually cannot be so high.

Unless typhoid bacteria are properly fixed and stained they do not reveal the presence of the flagella, seen in this micrograph.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochro-
mat

Eyepiece — Homal IV

Condenser — Watson Holos

Illumination — 500-watt lamp

Plate — Wratten M

Filter — G (orange)

Exposure — 45 seconds



PLATE XXVII. ANTHRAX BACTERIA IN PUS SMEAR, SHOWING THE CAPSULE
MAGNIFICATION 1700 \times

The bacillus of anthrax is a fairly large organism, usually connected in short chains. Ordinary staining methods do not reveal the presence of the gelatinous capsule which surrounds the cells and is responsible for the adherence in chain fashion.

In taking this micrograph, special attention was given to emphasizing the bacteria and their capsules and subordinating the pus cells.

Exposure Data

Objective - 1.5 mm. (120 \times) apochromat

Eyepiece - Zeiss #8 compensating

Condenser - Watson Holos

Slide stained with Negri body stain

Illumination - 5 ampere arc lamp

Plate - Wratten M

Filter - E (light red)

Exposure - 1 second



PLATE XXVIII. *TREPONEMA PALLIDUM*, IN SITU IN HUMAN KIDNEY
MAGNIFICATION 4100 \times

The causative organisms of many of our common diseases were identified around the early 1880's, but science looked in vain for that responsible for syphilis. It was only a scant thirty-five years ago that this corkscrew-like organism was found to be the cause of this dread disease. This seems strange, in the light of the ease with which it can be demonstrated now, as is evident from the micrograph.

In this view the organisms are seen in a section of a syphilitic kidney where they occupy all possible positions and are only occasionally in focus. While the micrograph is only an optical section, the blending of the non-sharp portions of the organisms into the plane of focus results in an approximation of three-dimensional effect.

Exposure Data

Objective - 1.5 mm. (120 \times) apochro-

mat

Eyepiece - Zeiss #8 compensating ocular

Condenser - Watson Holos, oiled

Section stained with Fontana's method

Illumination - 10 ampere arc lamp

Plate - Wratten Panchromatic

Filter - E (light red)

Exposure - 10 seconds

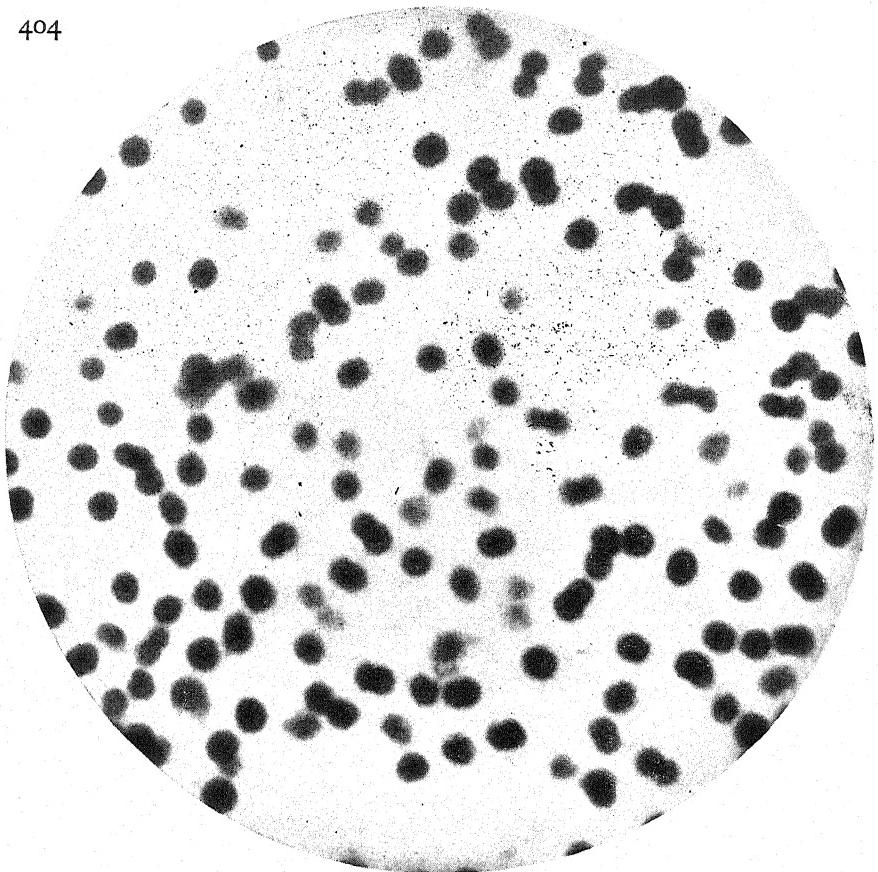


PLATE XXIX. MENINGOCOCCUS, SHOWING NUCLEUS AND CAPSULE
MAGNIFICATION 4500 \times

Bacteriologists have been divided in opinion as to the possibility of bacteria being nucleated. Ordinary staining methods do not furnish positive proof. In the first place, the organisms are so small that nuclear differentiation has not seemed possible although there is frequent evidence of a granulated nucleus in some of the larger species.

The micrograph shown here is one of the most positive proofs, in all probability, of a definite nucleus within the cytoplasm of the cell during active proliferation, at least. The meningococcus is extremely small, averaging around one micron in diameter. Stained with carbol-fuchsin it presents a uniform red appearance when examined visually. When photographed at a very high power, using the D line of the electric sodium lamp as the illumination source, not only a centrally placed nucleus is evident, but a capsule also, the presence of which is not suspected otherwise. It is difficult to bring these out in a reproduction as they show on the print. The reason for the differentiation lies in the greater absorption of the D line by the nucleus. The nucleus averages about .2 micron in diameter.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochromat	Illumination — Electric sodium lamp
Eyepiece — Homal IV	Plate — Wratten M Filter — none
Condenser — 1.4 N.A. aplanat, oiled	Exposure — 2½ minutes

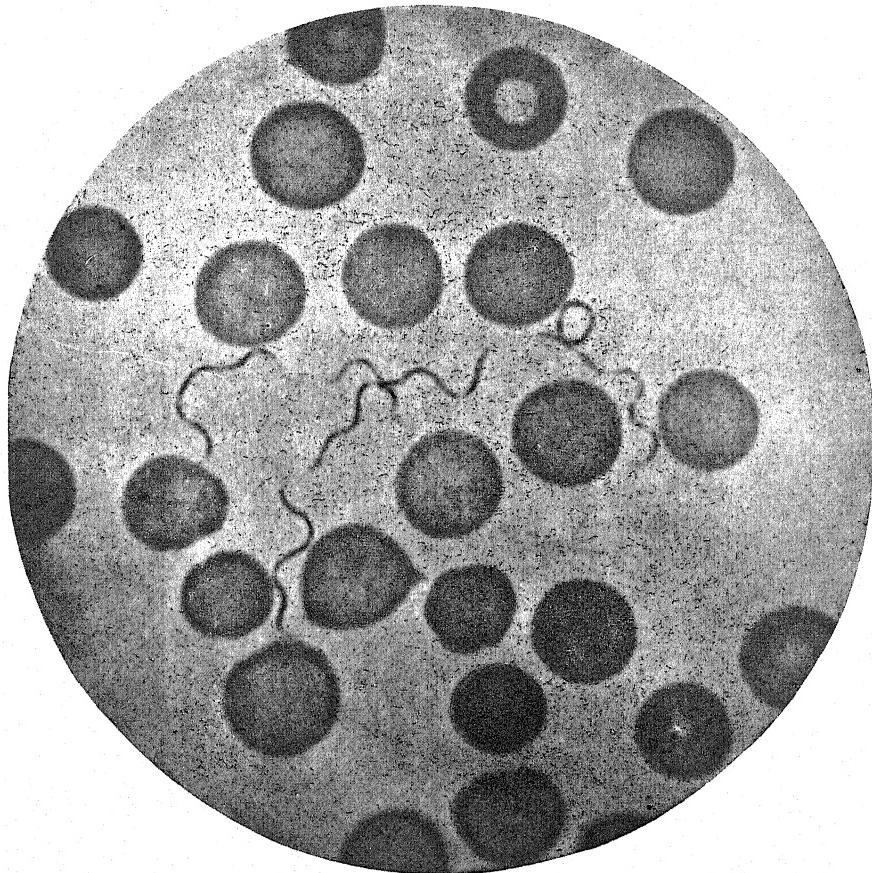


PLATE XXX. BORELLA RECURRENS
MAGNIFICATION 2500X

Here we have another illustration of the value of high magnification in depicting minute organisms. At the magnification employed for this micrograph, details within the bodies of the spirochaetes are clearly evident. While they would still register on the plate at a magnification of 1000 diameters, they would be relatively inconspicuous and probably not discernible to the unaided eye.

This is the organism which causes relapsing fever. Its minute size is appreciated by comparison with the size of the red blood corpuscles. In photographing a subject such as this, it is important that the organisms be in perfect focus, which generally means that larger objects, such as blood cells, are more or less out of focus.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochro-
mat

Eyepiece — Homal IV

Condenser — 1.4 N.A. aplanat, oiled

Blood smear stained with Giemsa stain

Illumination — 500-watt lamp

Plate — Wratten M

Filter — G (orange)

Exposure — 1 minute

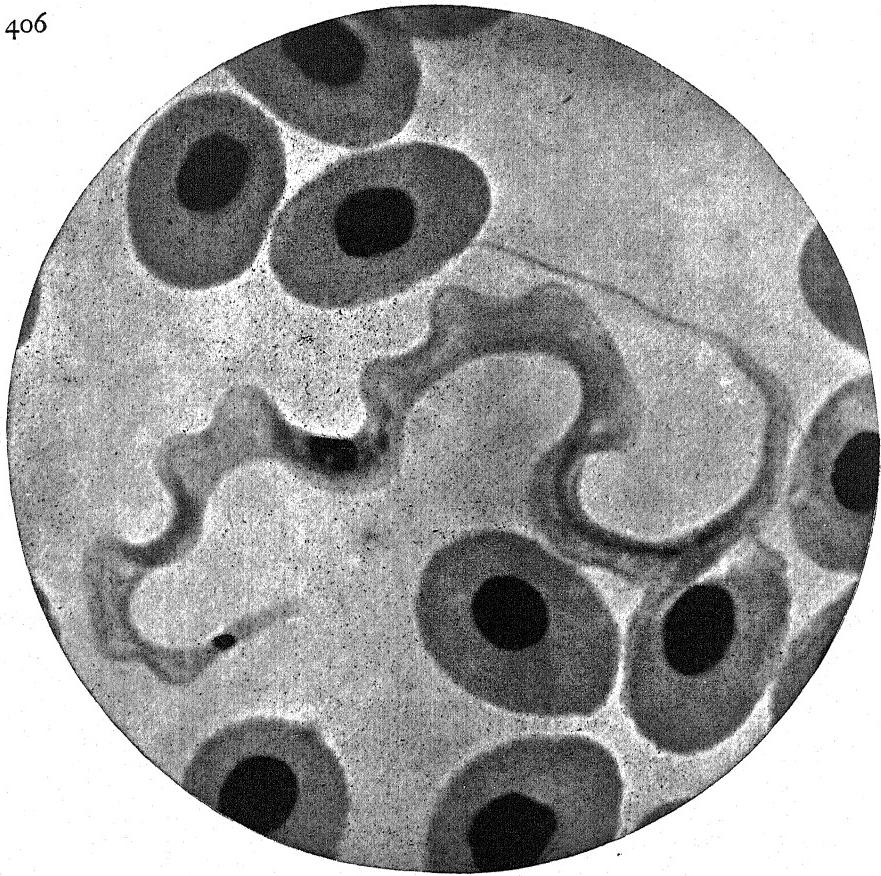


PLATE XXXI. *TRYPANOSOMA GRANULOSUM*
MAGNIFICATION 2500 \times

This high-power picture not only provides an easily interpretable view of a treponosome, but also illustrates a condition often encountered when using high-aperture lenses.

At first glance it might seem that a noticeable amount of fuzziness is present, suggesting a marked degree of empty magnification. But this is not the case. The objective was focussed on the flagellum, examination of which will show it to be sharply defined. Nearly everything else in the field is more or less out of focus, which is the true cause of the lack of sharpness.

It is apparent that high-aperture lenses possess practically no depth of focus; even a small fraction of a micron makes an appreciable difference in the sharpness of the image. In cases of the kind shown here, one must either attempt the best compromise focus or confine attention to some individual part of maximum interest, ignoring everything else.

Exposure Data

Objective - 2 mm. 1.4 N.A. apochro-
mat

Eyepiece - Homal IV

Condenser - 1.4 N.A., oiled

Specimen stained with Wright's stain

Illumination - 10 ampere arc lamp
Plate - Wratten M

Filter - B (green) plus G (orange)

Exposure - 4 minutes

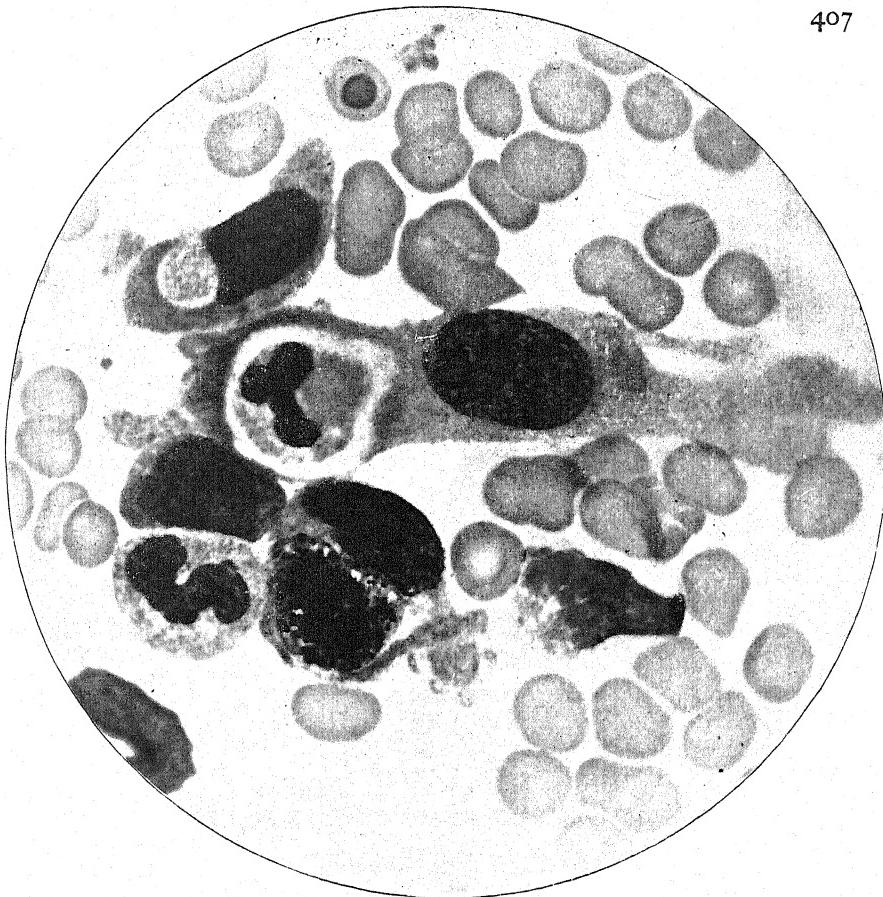


PLATE XXXII. HISTIOCYTES IN BLOOD

MAGNIFICATION 1300X

Although this micrograph has been included here as representative of the general field of hematology, it is so spectacular in what it depicts that it deserves special attention. Histiocytes are rare giant amoeboid cells that in certain blood diseases get into the blood stream. They are so cannibalistic that they not only eat the red blood cells but the large white cells as well. In the picture, the smaller individual at the top is seen in the very act of ingesting a red cell, which is only partly surrounded.

The white cells, known as polymorpho-nuclear leucocytes, are often called the policemen of the blood, for it is their function to destroy all undesirable foreign matter. This they do by eating it up, a process known as phagocytosis. But they cannot get rid of the histiocytes in this manner because the latter eat them instead. The large histiocyte in the center of the field has eaten a "poly" and is digesting it. The clear ring around the poly represents the degree to which the digestion has taken place. Large pseudopodia are to be seen on the histiocyte.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochromat

Eyepiece — Homal IV

Condenser — Watson Holos. oiled

Blood smear stained with Giemsa stain

Illumination — 500-watt lamp

Plate — Wratten M

Filter — E (light red)

Exposure — 7 seconds

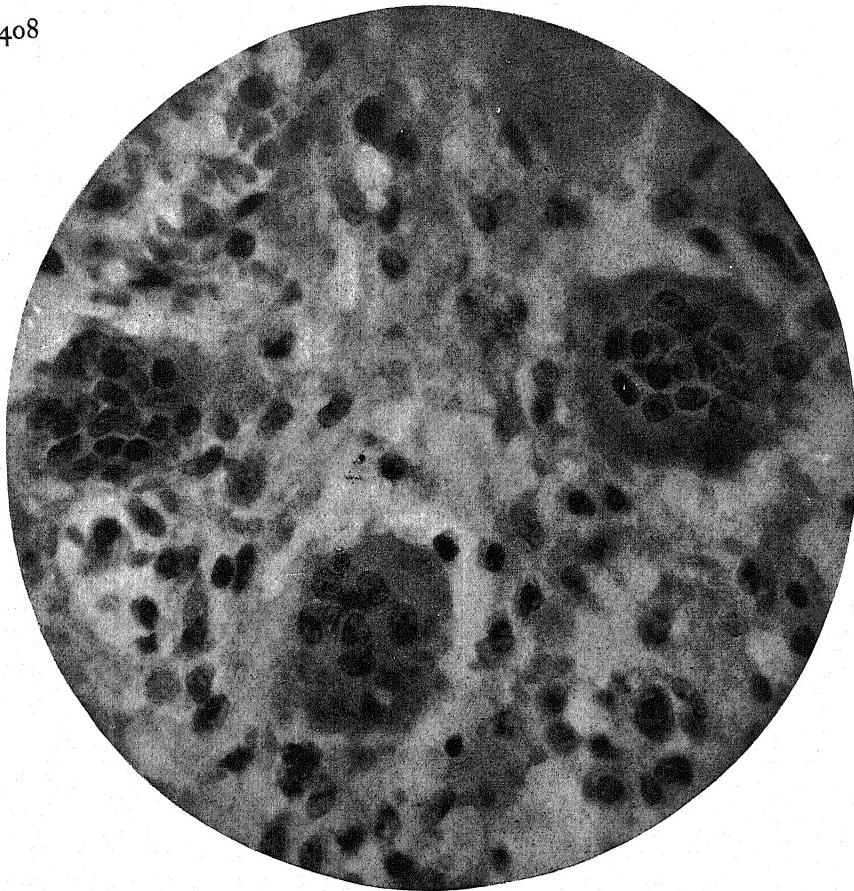


PLATE XXXIII. GIANT CELL SARCOMA
MAGNIFICATION 600x

The most prolific production of photomicrographs is to be found in the field of pathology, but probably in no other field is so much mediocre work done. This is not to be blamed on the apparatus, but on two controllable factors. One is the use of filters not suitable to the subject (usually too contrasty); the other is failure to employ critical illumination, with substantially full aperture. Reduced aperture in the illumination cone is not desirable in this class of work where differentiation is secured by staining.

Pathological and histological tissues should be portrayed along the lines illustrated by this micrograph. The nuclei of the cells should not be black, unless they are visually very dark in the section, and there should be no diffraction evident around the various structures.

Exposure Data

Objective — 8 mm. (20x) apochromat
Eyepiece — Homal I
Condenser — 1.4 N.A. aplanat
Illumination — 500-watt lamp
Section stained with hematoxylin and eosin

Plate — Wratten M
Filter — B (green)
Exposure — 30 seconds



PLATE XXXIV. GRAPHITE IN CAST IRON
MAGNIFICATION 100 \times

The cast-iron metallurgist is much concerned with the nature and dispersion of graphite in the metal. Cast iron usually contains around 3% to 3½% of carbon, only a small proportion of which is combined with the iron, as in steel. The balance is present as graphite. Large graphite flakes tend to lower the strength of the metal; hence the desire to obtain small flakes and a uniform dispersion.

When examining a specimen of cast iron for graphite, the surface must be highly polished to eliminate scratches, but it is not etched. The graphite then shows as black flakes against a mirror-like background, the metal itself.

Examining and photographing metals requires the use of vertical illumination and objectives which are corrected for use without a cover glass.

Exposure Data

Objective — 16 mm. (10 \times) apochromat

Eye-piece — Homal II

Vertical illuminator with plain glass,

10 ampere arc lamp

Specimen unetched

Plate — Wratten M

Filter — Wratten C (blue-violet)

Exposure — 1 second



PLATE XXXV. FERRITIC CAST IRON
MAGNIFICATION 300X

For examination of the matrix of cast iron it is necessary to subject the polished specimen to an acid etch which will attack the constituents differently and thus reveal the structure. This micrograph shows the appearance of a low-grade cast iron in which practically all the carbon has become graphite and none is left in combination with the iron. This not only results in excess graphite (the black areas) but lowers the strength of the metal, which is materially stronger when it has the proper percentage of carbon combined with it, as in steel.

All metals when examined under the microscope are seen to be composed of individual crystal grains. These grains are clearly evident in the micrograph. In this case they are called *ferrite* grains (i.e. meaning pure iron), whereas if the proper amount of carbon were present in them, they would be known as *pearlite* grains.

Exposure Data

Objective - 16 mm. (10x) apochromat

Eyepiece - Homal I

Vertical illuminator with plain glass,

10 ampere arc lamp

Specimen etched in 3% nital

Plate - Wratten M

Filter - Wratten #64 plus D (purple)

Exposure - 12 seconds

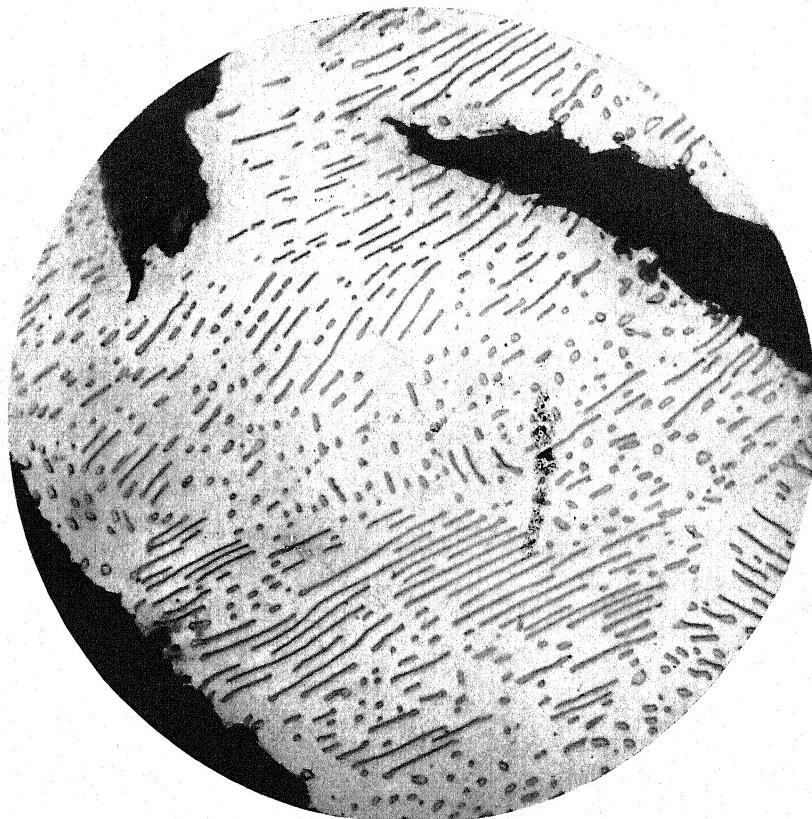


PLATE XXXVI. SPHEROIDIZED CAST IRON

MAGNIFICATION 2000 \times

Carbon which is combined with iron in steel and cast iron forms a compound known as iron carbide, or cementite. This occurs scattered through the grains, usually as thin parallel plates, as though the metal were laminated. Sometimes, however, it exists in small globules, as seen in this micrograph. As these globules are often quite small, it requires a high magnification to reveal them and the nature of their dispersion.

High-power metallurgical micrographs should show minute constituents, such as these, sharply outlined; the entire area of the picture should be evenly illuminated and in focus everywhere. To meet this latter condition, the surface of the specimen should be at absolute right angles to the optic axis.

Exposure Data

Objective - 2 mm. 1.4 N.A. apochro-
mat

Eyepiece - Homal IV

Plate - Wratten M

Specimen etched in 3% nital

Vertical illuminator with plain glass,
10 ampere arc lamp

Filter - C (blue-violet)

Exposure - 35 seconds



PLATE XXXVII. PEARLITE GRAIN IN CAST IRON

MAGNIFICATION 3000X

Extremely high magnifications often can be used to advantage in metallographic work. Structures which otherwise might escape detection are made evident. It is especially desirable that all unusual metal structures, new alloys, etc., be subjected to high-power analysis, for in this way new facts may be gleaned regarding them.

This micrograph shows an example of high magnification applied to the pearlitic structure of a coarse cast iron. It includes a portion of four grains. The lamina of the cementite plates run in different directions in each.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochro-mat

Eyepiece — Homal IV

Plate — Wratten M

Specimen etched in ammonium persulphate

Vertical illuminator with plain glass,
10 ampere arc lamp

Filter — C (blue-violet)

Exposure — 1 minute

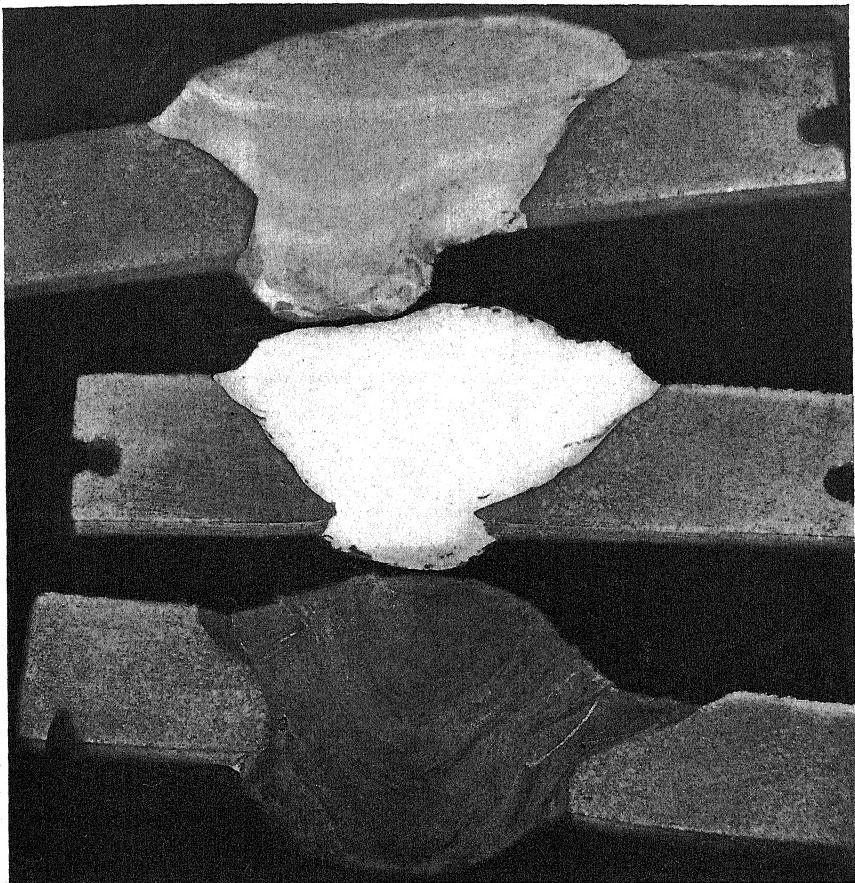


PLATE XXXVIII. EXPERIMENTAL WELDS ON STAINLESS CLAD STEEL
MAGNIFICATION 8x

Unusual problems frequently confront the metallurgist in connection with his constant search for improved metals. This micrograph shows the picture which was made as a permanent record of the relative quality of three different welds on a stainless clad steel. In this case the question was not one of strength — this must be determined by another kind of test — but of the relative resistance to acid attack on three different weld materials, and the effect of the welds on the structure of the adjacent steel.

It was therefore necessary that all samples be treated exactly alike. They were all embedded together, for this treatment, within a suitable matrix and handled as though they were one piece. The notches on the ends of the bars were for the purpose of identifying the individual samples in the micrograph.

This is an illustration of low-power metallographic work. In this case a special form of glass reflector is mounted under the lens, i.e., between the lens and the specimen, as vertical illuminators are not available for mounting above the large Planars.

Exposure Data

Objective — 75 mm. Planar
Illumination — 10 ampere arc with special plain glass mirror

Plate — Hammer Slow
Filter — 4-inch K-2
Exposure — 4 second

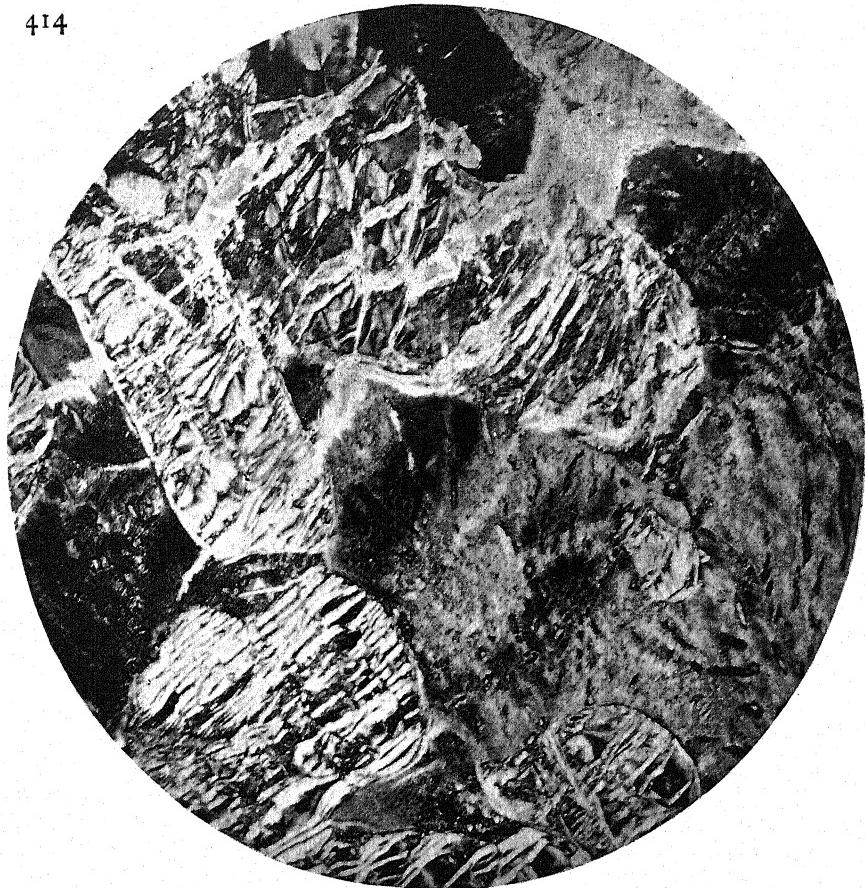


PLATE XXXIX. SERPENTINE ROCK SECTION
MAGNIFICATION 20 \times

Rock sections offer problems of an unusual nature to the photomicrographer in that they require polarized light to reveal the different minerals present. Visually the result of examination under polarized light is very satisfying, at least from an aesthetic viewpoint, but to translate all the beautiful effects seen into a black and white picture is quite another matter.

Color intensities as they affect the eye are often at variance with the extent to which they record on the sensitized plate. Another condition present is the extinction of crystals with crossed prisms which causes them to appear black in the extinction position, whereas they are actually transparent and possibly highly colored in other positions.

A serpentine rock, such as shown here, is less difficult to portray than many others, because the mesh structure of the altered olivene crystals and the alteration products of other minerals possess some pattern which can be reproduced in black and white, even under crossed prisms.

Exposure Data

Objective — 50 mm. Planar

Condenser — 5 cm. with 1 inch Nicol

Plate — Wratten M

Large 1 inch Abbe polarizing prism in tube back of lens, prisms crossed

Illumination — 500-watt lamp

Exposure — 2 seconds



PLATE XL. DIORITE DRIFT ROCK
MAGNIFICATION 22x

This micrograph illustrates what can usually be expected in the portrayal of rock sections where practically every field will possess one or more crystals in a position of extinction, no matter what the rotational angle may be.

Careful adjustment of the rotational position in this case has allowed a considerable portion of the plagioclase feldspar and the hornblende to appear in partial illumination, at least. Occasionally, when an excess of crystals occurs in an extension position, no matter what the rotational position may be, it is permissible to uncross the prisms slightly. This should never be done, however, when it is desirable to reveal the presence of isometric crystals. On the other hand, one should try to eliminate the presence of all anisotropic crystals in an extinction position when it is desired to concentrate attention on those which are to be identified by their non-polarization.

Exposure Data

Objective — 35 mm. Planar
Condenser — Spectacle lens with po-
larizer
Large Abbe prism in tube, prisms crossed

Plate — Wratten Panchromatic
Illumination — 500-watt lamp
Exposure — $\frac{1}{2}$ second

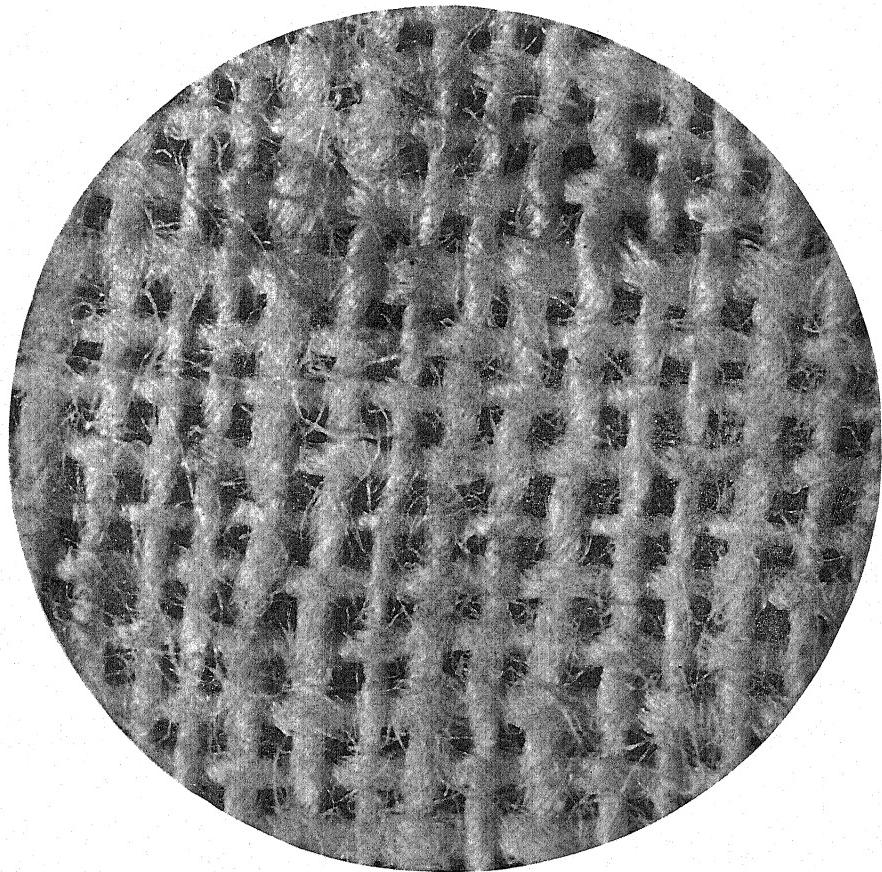


PLATE XLI. COTTON HANDKERCHIEF, SHOWN BY OBLIQUE TOP LIGHTING
MAGNIFICATION 20 \times

Pictures of textile fabrics at low magnification, taken with oblique top illumination, do not present any unusual problems to the photomicrographer. Most important is the lighting; this might require some slight modification in the angle of the light with respect to the optic axis, or the azimuth direction with respect to the weave, for each type of fabric. The most important requirements are to accentuate the weave itself and to bring out the individual fibers of which the thread is composed.

To the uninitiated, a picture such as this provides a better appreciation of the meaning of magnification than almost any other subject. There is always surprise manifested when it is pointed out that the magnification is only twenty times. The average guess puts it anywhere from one to several hundred times.

Exposure Data

Objective — 50 mm. Planar at F:9	Plate — Wratten M
Illumination — 5 ampere arc, without	Filter — none
collimating condenser	Exposure — 12 seconds
Fabric supported about $\frac{1}{2}$ " away from black background	

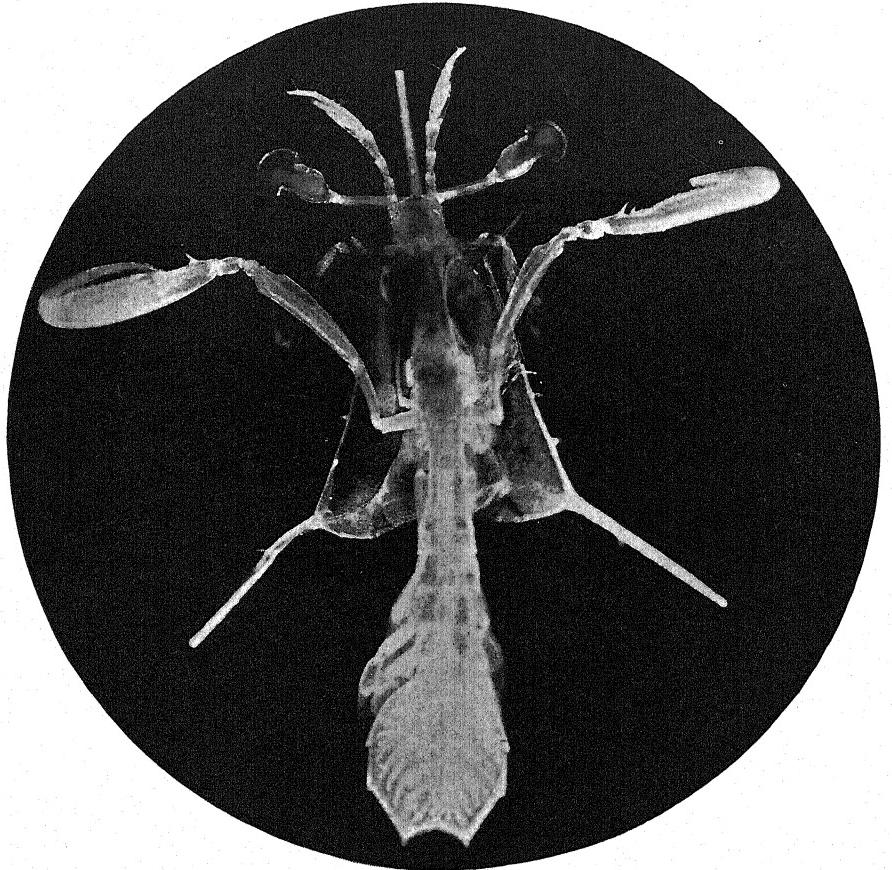


PLATE XLII. SQUILLA DESMARESTII, SHOWN BY DARK FIELD ILLUMINATION
MAGNIFICATION 12 \times

This beautiful animal, the alima stage of *Squilla desmarestii*, offers a fine subject for low-power dark field. In this case the dark field must be secured through the use of a central stop in a low-apertured condenser. Standard dark field condensers are designed for high-power work and cannot cover the field required for such a large object.

The considerable depth of the body of the specimen required the use of a long-focus lens to accommodate it and show every part in focus.

Exposure Data

Objective — 50 mm. Planar slightly stopped	Condenser — 1.4 N.A. aplanat, with top removed and central stop below
Filter — E (deep orange)	Illumination — 500-watt lamp
Plate — Wratten M	Exposure — 5 seconds
Object mounted in fluid, without pressure, not stained	



PLATE XLIII. *NOSTOC*, SHOWN BY DARK FIELD ILLUMINATION
MAGNIFICATION 475 \times

This minute blue-green alga, only a little higher in the scale of life than the bacteria, is a beautiful subject for dark field work. The minute coccus-like spherical cells, joined together in chains, simulate fairy necklaces as they glisten in the brilliant light of dark field illumination. The specimens shown here were mounted in fluid and hence are not all on the same plane. Even with the use of a lower-apertured objective not all could be in focus at the same time, but the results are suggestive of a three-dimensional picture, for the eye itself can focus upon but one plane at a time.

The micrograph is typical, otherwise, of dark field work. The only problem of an unusual nature lies in obtaining the suggestion of translucency in the cells rather than a brilliant white, devoid of detail. This is done by working for softness, rather than contrast in the negative.

Exposure Data

Objective — 16 mm. (10 \times) apochromat

Eyepiece — Homal I

Condenser — Parabaloid

Magnification obtained partly through extra-long bellows extension

The specimen was unstained, mounted in fluid

Illumination — 10 ampere arc

Plate — Wratten M

Exposure — 12 seconds

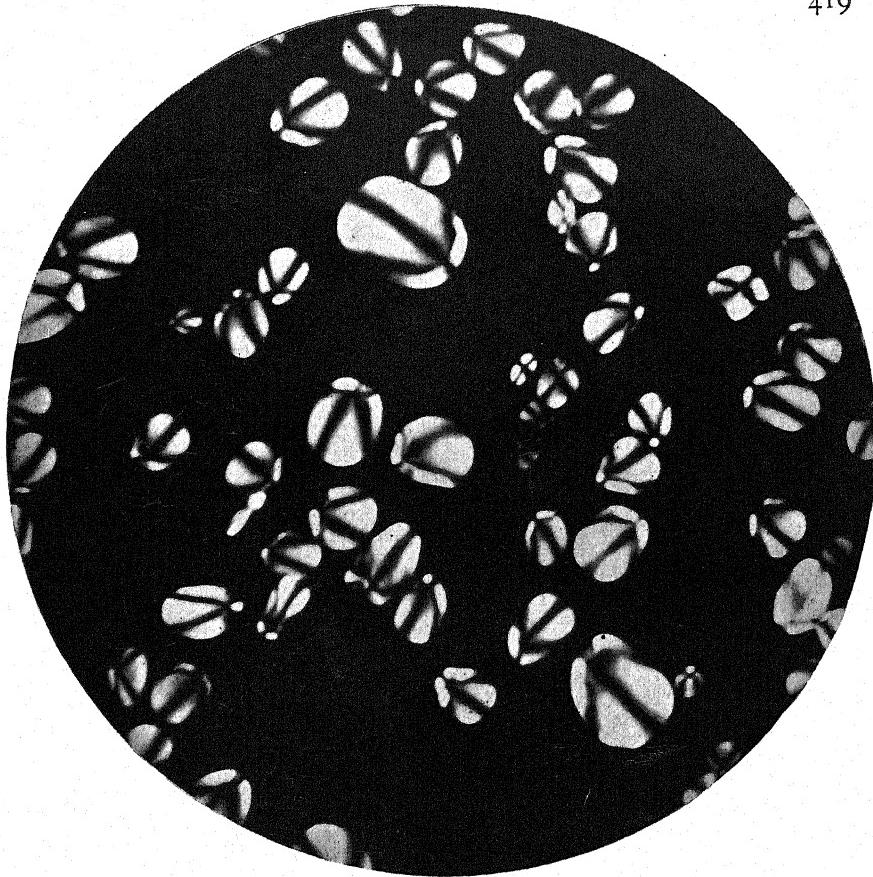


PLATE XLIV. TOU-LE-MOI STARCH, SHOWN BY POLARIZED LIGHT
MAGNIFICATION 160 \times

Starches are easily identified in microscopical preparations by means of polarized light. With the polarizing prisms crossed, the background is dark, but any object possessing birefringence, which may be in the field, appears illuminated due to the interference set up by repolarization. Starches, being in this class, show brightly against the black background, each grain marked with a black cross. The arms of the cross connect at the hilum, or core, of the grain, which in most starches is not centrally located.

Tou-le-moi starch grains are among the largest known, and therefore serve well to demonstrate the typical appearance of starch under polarized light. In taking a micrograph such as this, the exposure and development should be correct, so that the grains will stand out brilliantly white, on a black background.

Exposure Data

Objective — 16 mm. (10 \times) apochromat
Eyepiece — Homal I
Condenser — 1.4 N. A. aplanat
Polarizers crossed

Illumination — 500-watt lamp
Plate — Wratten M
Exposure — 35 seconds



PLATE XLV. RICKETS IN RAT JOINT, SHOWN BY INFRA-RED LIGHT
MAGNIFICATION 18x

The value of infra-red light in the photography of some types of tissue is well brought out in this micrograph. The section, cut in celloidin, was mounted unstained, in glycerin. This necessitated the use of the microscope in the vertical position. Comparative tests with light in both the visible and ultra-violet regions demonstrated conclusively the superiority of the infra-red in this particular case. Infra-red brought out structural details not made evident by either of the other light bands. Information also is gained which cannot be secured by standard staining methods.

Exposure Data

Objective — 20 Planar	Filter — Schott infra-red
Illumination — 500-watt lamp, with	Plate — Eastman Spectrographic #I-P
Köhler system	Exposure — 8 seconds
Condenser — 2 cm. spectacle lens	



PLATE XLVI. VANADIUM DISPERSION IN VANADIUM CATALYST, SHOWN BY TRANSMITTED ULTRA-VIOLET LIGHT 275 W.L.
MAGNIFICATION 80X

One of the most valuable contributions of the ultra-violet microscope to science has been in the identification of various constituents in a given specimen, through a difference in their relative transmission of ultra-violet light. This micrograph is a typical example. The problem was to determine the dispersion of the vanadium compound in a catalyst pellet. Was it uniformly distributed throughout, segregated, or otherwise?

By ordinary methods nothing could be ascertained; special methods to differentiate the vanadium were necessary. A section (about 15 microns thick) was photographed by an abnormally long exposure, to over-expose all constituents *not* vanadium. The latter is so absolutely opaque to ultra-violet that even an exposure of several hours will not make an impression on the plate, where even a minute quantity of vanadium is present. Hence, we have obtained a silhouette picture, the black representing vanadium, the white, all other constituents. A normal exposure would have shown structure in the non-vanadium constituents.

Exposure Data

Taken on the Zeiss Ultra-Violet outfit, with the object mounted in Vinylite on a quartz slide, and quartz optics throughout.

Illumination — cadmium spark, 275 m μ
Plate — Hammer Slow

Exposure — 20 minutes (normal for detail, 1 minute)

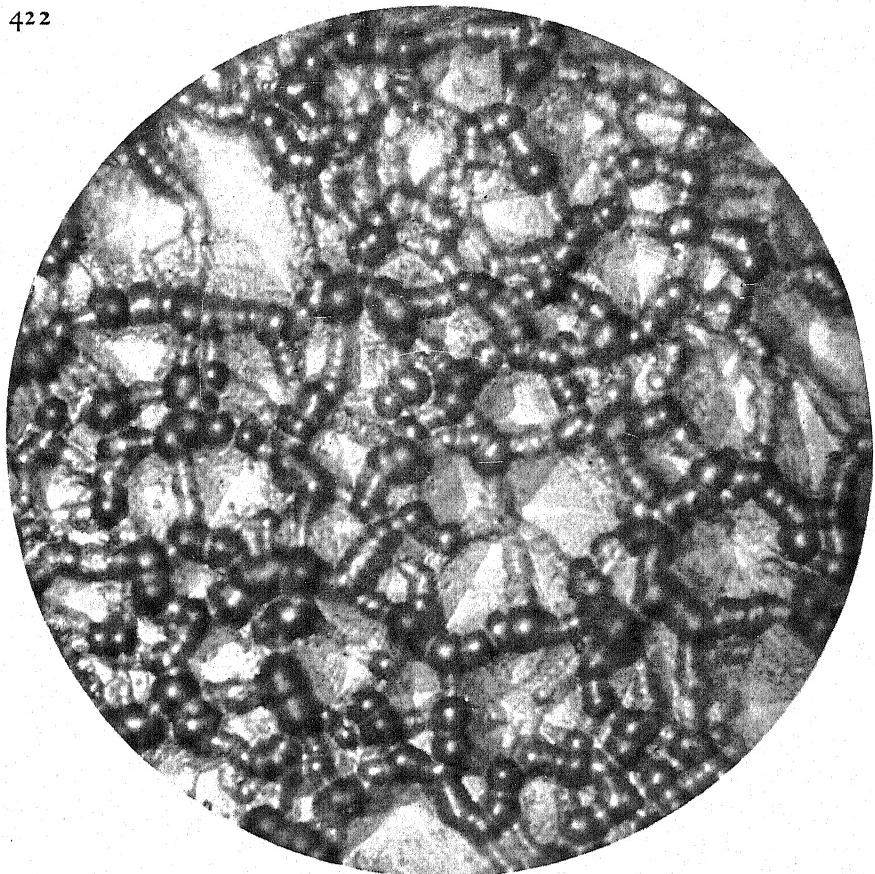


PLATE XLVII. FROSTED SURFACE OF ELECTRIC LIGHT BULB, ILLUMINATED BY
INCIDENT LIGHT
MAGNIFICATION 1000 \times

This object offers photomicrographic problems of a very unusual nature. The frosting is done on the inside of the bulb, every part of which is concave or compositely curved. The etch is very fine, requiring high magnification to reveal its nature, and two types of etch are present — pyramidal elevations with flat angular sides and a secondary structure in the crevasses, consisting of rounded depressions. Thus the total depth of focus required is considerable. Lastly, the material is transparent glass. The illumination must be fairly symmetrical, to avoid deep shadows.

In one respect the micrograph requires explanation, that the true nature of the rounded areas may be understood. At times they give the impression of rounded elevations, whereas they are cavities. The reason is that, being glass, the reflections from a negative sphere can be identical with those of a positive sphere. In a monocular view there is no way of distinguishing them apart.

Exposure Data

Zeiss Epi-W Condenser, with special attachment for external illumination.

Objective — 21 \times Epi

Eyepiece — Homal III

Illumination — 10 ampere arc

Plate — Wratten M (full 6½ x 8½)

Filter — none

Exposure — 50 seconds

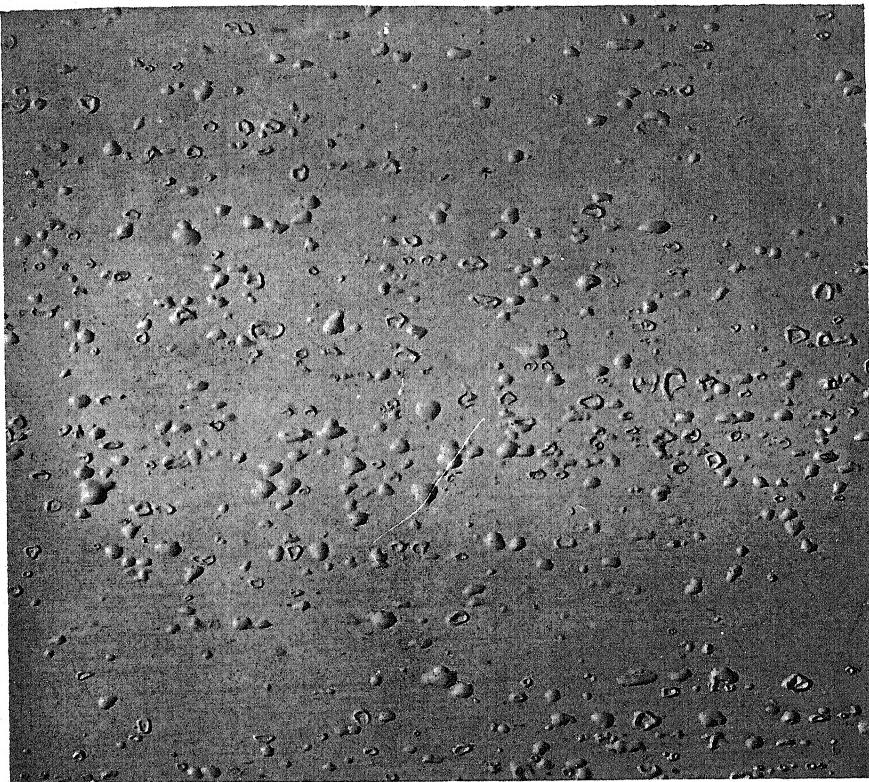


PLATE XLVIII. PAINT TEST PANEL
MAGNIFICATION FULL SIZE

Research departments of large manufacturing concerns are continually making tests of products under exaggerated service conditions, with a view to establishing causes of failure and the average length of life to be expected. To supplement the written records of such tests, it often adds materially to the data to attach a picture or photomicrograph which visualizes the appearance of the product after test. This picture shows one of a series of paint panels after going through a specific routing of conditions. From the standpoint of magnification, the picture represents the borderline between commercial photography and photomicrography, as the subject has been neither enlarged nor reduced, in the taking of the picture.

The paint on the panel was white, but the color not being a factor in this case, it was allowed to appear appreciably dark, in order to emphasize the presence of the small blisters, the cause of final failure. To be of real value, a picture of a surface such as this, must suggest a three-dimensional aspect. This is accomplished entirely by manipulation of the lighting.

Exposure Data

Objective — 7-inch Dagor

Filter — none

Illumination — one 400-watt lamp

Exposure — 1 second

Plate — Hammer Slow

Light very oblique to surface and far away, to give even illumination. The room was well lighted by daylight, in addition to artificial light.

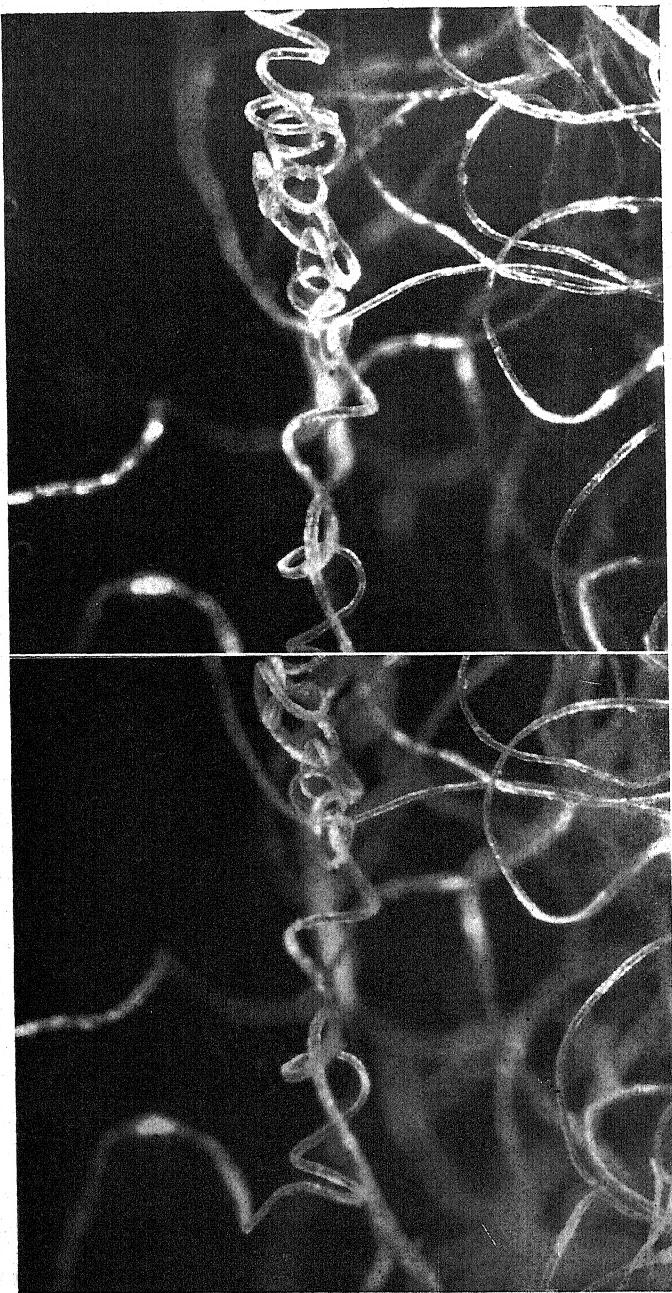


PLATE XLIX. STEREO-MICROGRAPH, WOOL FIBER, SHOWING NATURAL CURL.

MAGNIFICATION 50x

Exposure data — Same as Plate L.

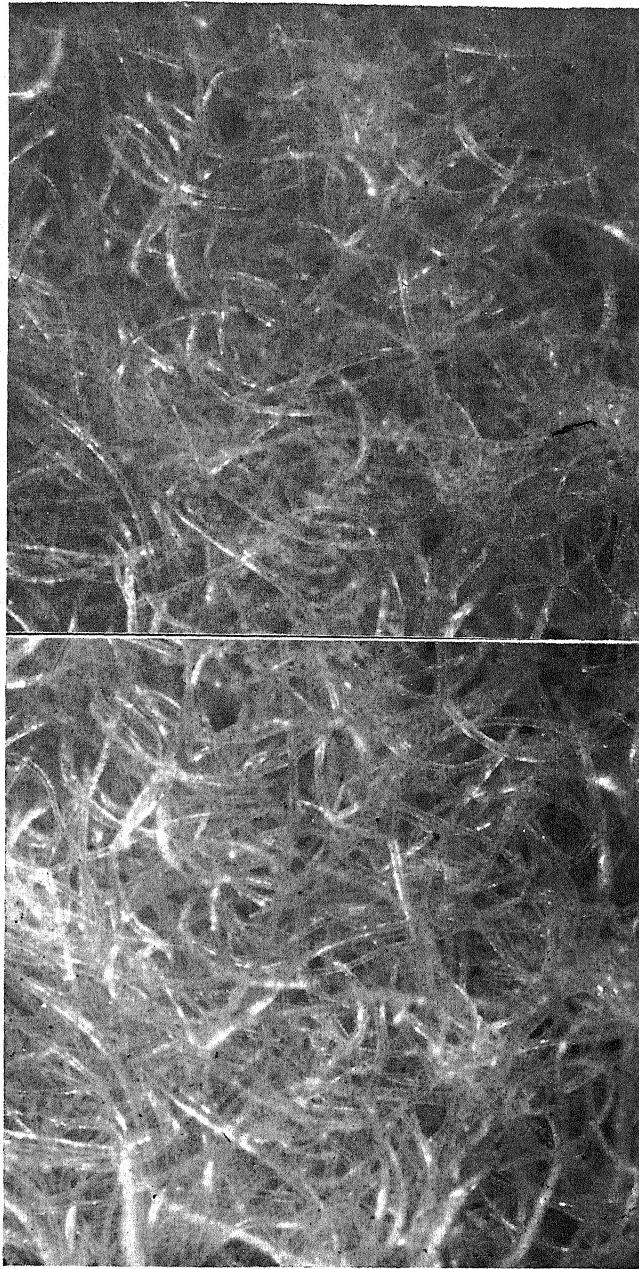


PLATE L. STEREO-MICROGRAPH, WOOL FELT
MAGNIFICATION 50x

Exposure Data

Objective — 20 Planar, at F:11
Illumination — 10 amp. arc, mirror reflected
Plate — Eastman Polychrome
Filter — none
Exposure — 12 seconds (each)

Taken by means of special stereo tilting stage

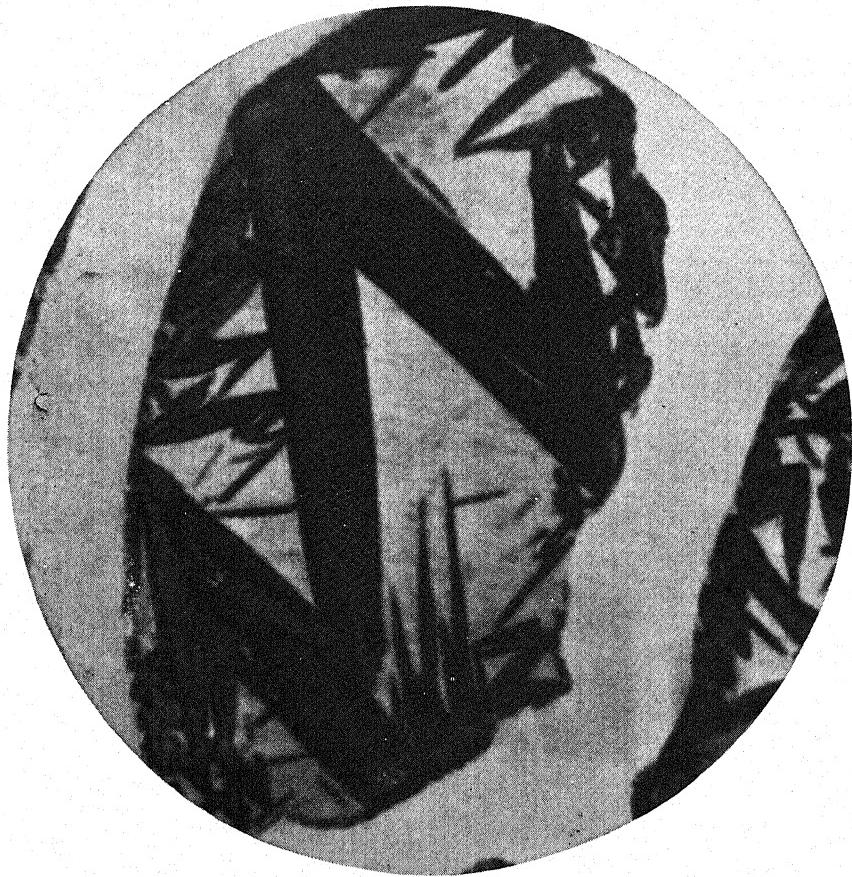


PLATE LI. MARTENSITE IN CHILLED CAST IRON
MAGNIFICATION 4500 \times

This shows an island of martensite in the chill of a nickel-chromium cast-iron car wheel. The area around the martensitic nodule is a solid solution of iron carbide in iron. Martensite represents in this case the initial stage of complete crystallographic orientation into ferrite, the associated carbide plates and excess carbon, as graphite. Chilling has arrested the process, producing martensite, the hardest constituent possible in an iron-carbon metal, excepting pure carbide, Fe₃C.

Exposure Data

Objective — 2 mm. 1.4 N.A. apochromat

Eyepiece — Homal IV

Specimen etched in 3% nital

Light — 10-ampere arc

Vertical illuminator with plain glass

Plate — Wratten M

Filter — C (blue-violet)

Exposure — 2 minutes

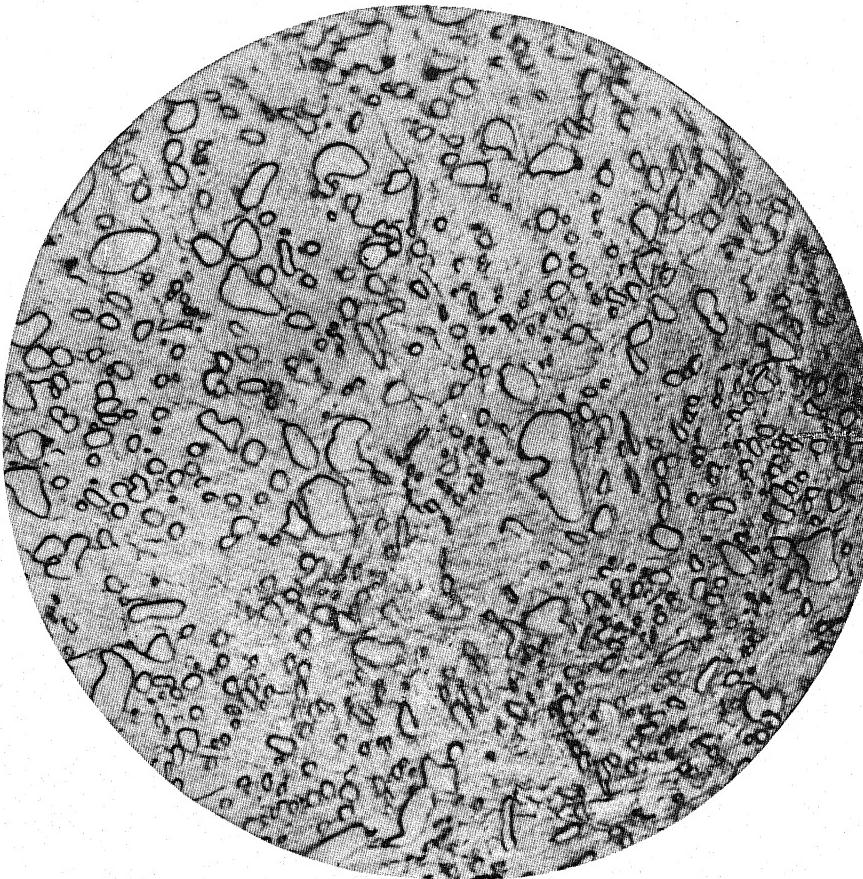


PLATE LII. S.A.E. 1095 DRILL-ROD STEEL
MAGNIFICATION 2000 \times

This hypereutectoid steel rod proved to be very hard machining. Microscopical examination of it showed it to be incompletely normalized, the matrix not being completely pearlitic, although the excess carbide (the clear islands) have been precipitated out. The residual hardness is due to the condition of the matrix.

Exposure Data

Objective - 2 mm. 1.4 N.A. apochromat	Light - 10-ampere arc
Eyepiece - Homal IV	Vertical illuminator, plain glass
Plate - Wratten Metallographic	Filter - Wratten B (green)
Specimen etched in 3% nital	Exposure - 17 seconds

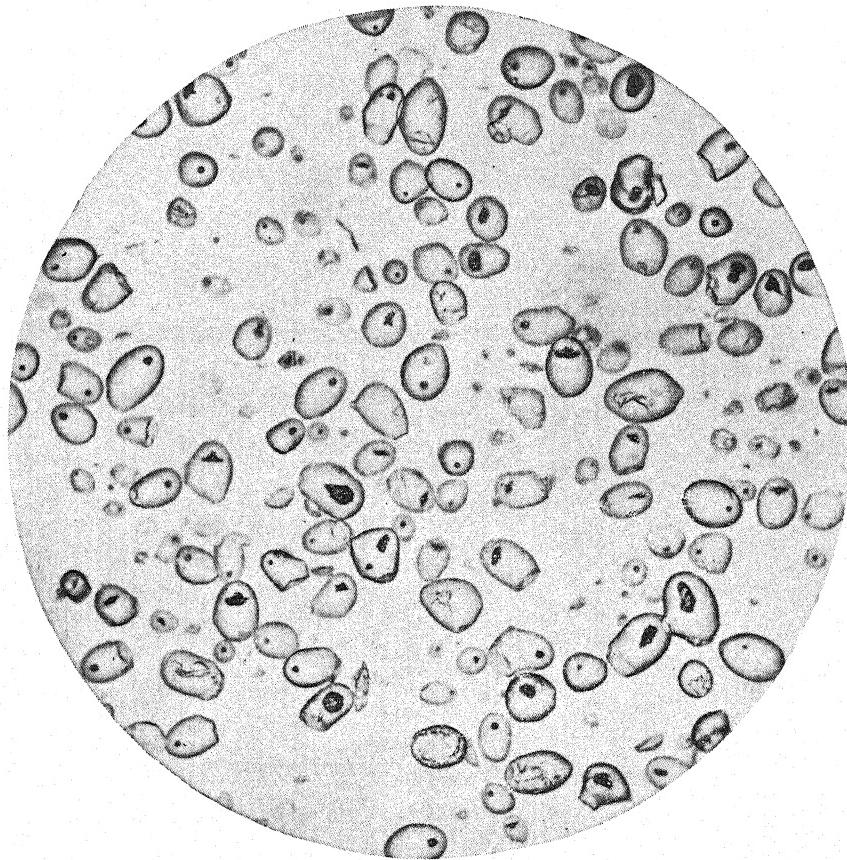


PLATE LIII. SAGO STARCH
MAGNIFICATION 200 \times

Unstained, mounted in a medium with a refractive index of 1.65.

Exposure Data

Objective — 10 \times apochromat
Eyepiece — Homal I
Condenser — 1.4 N.A. aplanat
Illumination — 500-watt lamp

Plate — Panatomic X
Filter — G (orange)
plus H-Lt (Light blue)

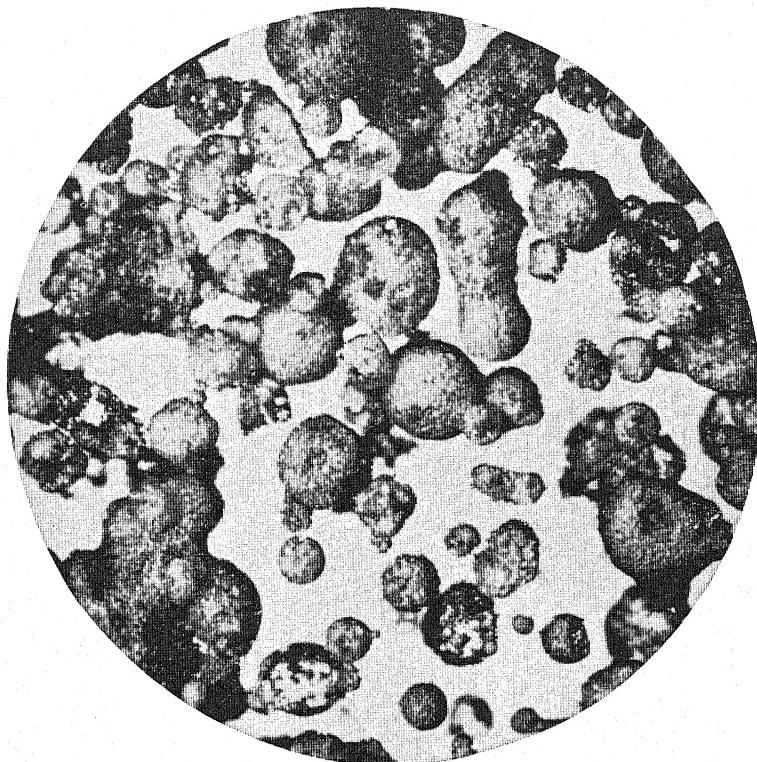


PLATE LIV. POWDERED SOLUBLE COFFEE
MAGNIFICATION 60 \times

Showing a type of object requiring a double exposure. The globules are hollow and dark brown in color and with transmitted light would photograph black. With top illumination they show very little differentiation from the background. Hence an exposure by transmitted light to provide a clear background, followed by a short exposure, oblique top illumination with a 5-ampere arc light, provides an ideal picture of the granules as they should appear.



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